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Waste Water

Evaluation and Management

Edited by Fernando Sebastián García Einschlag

WASTE WATER - EVALUATION AND MANAGEMENT

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Notice

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Meet the editor

Fernando S. García Einschlag was born in La Plata (Argentina) in 1969. He studied biochemistry at the University of La Plata and got his degree in 1995. From 1995 to 2001 he worked on his PhD Thesis "Photodegradation of nitroaromatic compounds by the UV/H2O2 process" at INIFTA (University of La Plata). In 2001, through a DAAD grant, he made a post-doc stay with Prof. Dr. André M. Braun and Prof. Dr. Esther Oliveros at the Karlsruhe University (Germany). During the stay in Germany he started working on the application of Fenton systems for waste water treatment. From 2005, he is a member of CONICET and since 2008, he is a professor at the La Plata University. His scientific work is mainly related to issues such as the evaluation of the efficiency of Advanced Oxidation Processes; the application of mathematical tools for kinetic and spectral analysis; and the development of simplified models for the quantitative description of complex reaction mechanisms. He is an author of 20 peer-reviewed publications in international journals.

Contents

Preface

Wastes are materials generated as a result of numerous anthropic activities and should be disposed to prevent environmental pollution which affects human health and contributes ecosystem degradation. Most of the environmental problems, which we are dealing with, are a result of improper management of wastes. In particular, fresh water resources are under serious stress throughout the globe. Water supply and water quality degradation are global concerns.

Anthropogenic pollution of natural waters constitutes of many substances and many water bodies receive a varied range of waste water from point and/or non point sources. Hence there is an increasing need for better tools to asses the effects of pollution sources and prevent the contamination of aquatic ecosystems. The reduction of risks to the aquatic systems requires the characterization of waste waters, the analysis of the impact of waste water on freshwaters and the reduction of contaminants through the application of appropriate policies and measures. Therefore, the development of improved monitoring tools, impact evaluation approaches and treatment technologies are active research fields.

The major sources of wastewater can be classified as municipal, industrial and agricultural. In order to evaluate the impact of these types of waste waters on the aquatic ecosystem, monitoring is necessary because wastewater is discharged back into surface. To ensure the effluent water quality, some global parameters such as biological oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC) and total suspended solids (TSS) are usually measured. In addition to the latter indicators, toxicological assessment of environmental samples is necessary for a proper monitoring of wastewater quality. On the other hand, the use of models for predicting effluent indicators can provide effective guidance for the operation of waste water treatment plants.

This multi-authored book takes a look at various aspects concerning the evaluation and management of waste water. The book chapters, heterogeneous in nature, were invited by the publisher and the authors are responsible for their statements. The accuracy of each chapter was checked by the authors through proof reading stages. The book covers a wide spectrum of issues related to waste water monitoring, the evaluation of waste water effect on different natural environments and the management of water resources. The reader will find current information and data obtained in studies undertaken to assess the quality of waste waters and the impact of pollution sources on natural water bodies. The chapters are basically organized in three sections. In the first section, chapters are mainly focused on methods for monitoring parameters of waste water quality. The second section includes several case studies dealing with the evaluation of the effects of waste water on different regions in the world. The final section covers topics related to waste water management and environmental policies.

We hope that this publication will be a useful document for students, professionals and researchers of various disciplines related to waste water evaluation and management. The book is a result of efforts by many experts from different fields. I would like to acknowledge the authors, who are from different countries, for their contribution to the book. I wish to offer special thanks to Prof. Katarina Lovrecic for her exceptional help throughout the preparation process.

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Part 1

Waste Water Quality Monitoring

1

The Use of Methods of Environmental Analysis and Ecotoxicological Tests in the Evaluation of Wastewater

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1. Introduction

The term "new" environmental pollutant covers all anthropogenic contaminants that have been released in the environment over the last few decades. Research into their fate and impact on the environment has started relatively recently. The group of new environmental pollutants consists of persistent organic pollutants (POPs) including DDT, polychlorinated biphenyls, polycyclic aromatic hydrocarbons and a wide range of organic pesticides. This group has recently been expanded with polybrominated flame retardants, drugs, detergents, and personal hygiene products. Chemicals, used everyday in homes, industry and agriculture, can enter the environment in wastewater. According to the United Nations Organization 884 million people do not have access to safe drinking water, and over 2.6 billion people do not have access to proper sanitation systems. On July 28, 2010, the UN General Assembly passed a resolution supporting water as a human right and urging governments and international organizations to address this important human right.

Interest in the presence of pharmaceuticals, personal care products, and endocrine disruptors in the environment has increased significantly over the last decade. Pharmaceuticals and synthetic musk compounds in personal care products (PPCPs), are groups of organic micropollutants which are routinely detected in surface water, ground water and even in drinking water (McClellan and Halde, 2010). Hundreds of tons of these compounds are dispensed in communities every year. PPCPs are regarded as being potentially hazardous compounds as some are ubiquitous, persistent and biologically active compounds with recognised endocrine disruption functions (Kasprzyk-Hordern et. al., 2002). Many pharmaceuticals, upon administration, are degraded in the body and may even become inactive, but others, often those applied externally, excreted or not absorbed fully, can leave the body in their active forms. (Roberts &Thomas, 2006). The others only slightly transformed or even unchanged often conjugated to endogenous substrate (e.g. glucuronic acid, sulfuric acid, acetic acid to polar molecules. (Roberts and Thomas, 2006). These conjugates are easily cleaved during sewage treatment and, thus, several pharmaceutically active compounds (PhACs) are discharged almost unchanged from municipal sewage treatment plants (STPs) into the receiving waters (Heberer, 2002). The wide range of trace chemical contaminants persisting in municipal wastewater after conventional treatment includes inorganic compounds, heavy metals, persistent organic pollutants like endocrine disrupting compounds, pharmaceutically active compounds; personal care products (PPCPs), disinfection by-products and many other complex compounds persist through the waste water treatment process. As a result, terrestrial and aquatic organisms can be exposed to these compounds when treatment by-products are subsequently reintroduced into the broader environment. Exposure can be associated with effluents from waste water treatment plants (WWTPs) leach from landfills, and runoff or leach from land that has received applications of reclaimed waters and/or biosolids. Thus, a number of PPCPs are widely detected in water resources throughout the world (Edwards et al., 2009; Lapen et al., 2008; Fatta-Kassinos et al., 2010). From reasons mentioned above is very important to monitor large spectra of chemicals not only in biosolids, which represents inevitable by-products of WWTPs, but in reclaimed water from municipal wastewater treatment. Waste water treatments produces large amount of sewage sludges/biosolids. Part of them is after treatment process used as fertilizer (Jones-Lepp & Stevens, 2006). The Sewage Sludge Directive 86/278/EEC seeks to encourage the use of sewage sludge in agriculture and to regulate its use in such a way as to prevent harmful effects on soil, vegetation, animals and man. On the other hand sludge could be posing a risk for environment they tends to concentrate heavy metals and organic but its, however, rich in nutrients such as nitrogen and phosphorous and contains valuable organic matter. Directive 86/278/EEC sets limit values for seven heavy metals, for some congeners of PCBs and AOX (Absorbable organic halides) other organic compounds and ecotoxicity tests arent′ required The European Commission is currently assessing whether the current Directive should be reviewed – and if so, the extent of this review (Council directive, 1986).

In the 1980´ and 90´the focus on the analysis of sewage sludge meanly deal with pathogens, inorganic (metals), polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) (Alcock et al.; 1996 Eljarrat et al., 2001). In 21TH century analysis of these matrices is not target only for selected traditional analytes but new xenobiotics as pharmaceuticals and musk compounds in sewage sludge are reported (Herren & Berset 2000; Zorita et al., 2009; McClellan & Halde, 2010). Ecotoxicological evaluation of sewage sludge as integral part of environmental analysis started to appear in this century sparsely (Park et al. 2005, Domene et al., 2008). Chemicals analysis is integral part of water quality evaluation but to predict effect of this water or used sewage sludge on environment is necessary to use some other possibilities. Useful toll for this could be biotests. Global evaluation of wastewaters should include ecotoxicological tests to complete the chemical characterization. The integrated assessment of biological effects of wastewater discharges in the ecosystems is relevant and ecotoxicity tests are referred as extremely useful tools for the identification of environmental impacts (Mendonca et al., 2009).

1.1 Pharmaceuticals

Pharmaceuticals are biologically active substances. It is documented that drug production and consumption are increasing worldwide, both in human and veterinary medicine. The consequence of this trend is the increased input of these substances into the environment, where they can exist in their original or metabolized form and can have significant impacts on natural ecosystems (Halling-Sörensen, 1998). The hazardous source of pharmaceuticals represents agriculture, predominantly livestock production. Growth stimulants and antibiotics used as prophylaxis drugs entered ecosystem in faeces and urine as fertiliser without possibilities degradation on less active metabolites in WWTP (Koschorreck et al., 2002). Biodegradation in digestive system of animals it is possible, but not for all substance. In study (Halling-Sorensen et al., 1998; Hirsch et al., 1999) was demonstrate, that 30−90 % doses of antibiotics enter he environment in origin form. At the end Pharmaceuticals are specially target on elimination of pathogenic organisms, in this case they could decrease number of microorganisms in WWTP and affect treatment process of water treatment. Antibiotics were recently classified as a priority risk group due to their high toxicity to algae and bacteria at low concentrations and their potential to cause resistance amongst natural bacterial populations Hence, antibiotics in surface water have the potential to disrupt key bacterial cycles/processes critical to aquatic ecology (nitrification/denitrification) or agriculture (soil fertility) and animal production (rudimentary processes) (Watkinson et al., 2007; Watkinson et al. , 2009).

	Diclofenac Sodium	Ibuprophen sodium	Ampicillin	Penicillin G
CAS	15307-79-6	31121-93-4	69-53-4	61-33-6
Physical state	White or slightly yellowish crystalline powder, slightly hygroscopic Sparingly soluble in water, freely soluble in methanol.	White crystalline powder. Sparingly soluble in water, freely soluble in acetone, methanol, ether.	White powder, slightly hygroscopic. Soluble in water, insoluble in alcohol, oils.	White powder. Soluble in water, insoluble in alcohol, various oils and paraffin's.
Molecular weight (g/mol)	318.13	228.26	371.39	334.39
Melting point (°C)	278-279	75−78	324.85	243.10
Boiling point (°C) at 25°C	412.00	157.00	622.61	565.30
Water solubility (mg l^{-1}) at 25°C	50.00	57,90	439.30	210.40
Henry's Law Constant (atm.m^3.mol^{-1})	$5.29.10^{-9}$	$1.23.10^{-6}$	$2.97.10^{-16}$	$1.88.10^{-13}$
Log K_{OW}	1.56	~ 1.00	1.45	1.83
Log K_{OC}	2.92	2.59	2.73	2.63

Table 1. Physico-chemical characteristics of pharmaceuticals

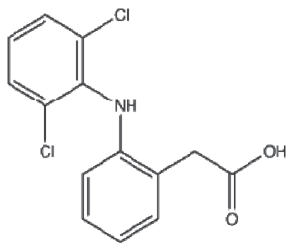
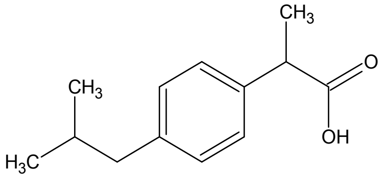
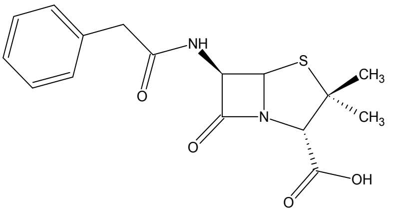
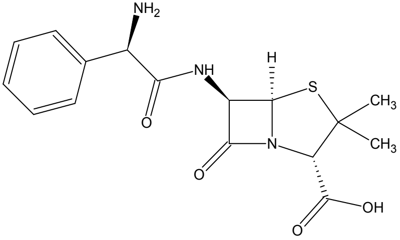
	
Diclofenac	*Ibuprofen*
	
Penicilin G	*Ampicillin*

Fig. 1. Structural formulae of selected pharmaceuticals

Since pharmaceuticals are designed with the intention of performing a biological effect, this concept may be very important for these specific substances in terms of fate and effects toward non target organisms in the environment, even at low concentrations. The food chain of some organisms is affected and pathogenic organisms can develop their resistance against these substances. Another important problem is presented by the negative impact on bacterial microflora in biological cleaning processes. Pharmaceuticals are classified as the so-called "microcontaminants", which have been causing significant concern within the environmental area during past years. (Beausse 2004; Gómez et al. ,2006)

The most pharmaceuticlas are not completely removed in the wastewater treatment plant (Beausse, 2004; Gómez et al., 2006; Ramirez et al., 2007). They are partially eliminated by sorption to wastewater sludge or by biodegradadion. The elimination level differs in dependence on the substance type and process conditions. The elimination of antibiotics from the wastewater treatment plants can be also affected by sludge age. Non-steroidal anti-inflammatory drugs (NSAIDs) with analgesic, antipyretic and anti-inflammatory effects such as diclofenac, ibuprophen and ketoprofen are one of the most widely used. It also belongs to the most frequently detected pharmaceuticals in the water-cycle in Europe. Antibiotics are the important group of the drugs affecting the environment. They are classified as biological, semisynthetic or synthetic substances. They are characterized by their selective toxicity against bacteria and they can be used to treat various infections. More than one hundred antibiotics are presently used. The primary classification of antibiotics is based on their effects and differentiates them on bactericidal and bacteriostatic. (Yang & Carlson, 2003; Kay et al., 2005).

1.2 Musk compounds

Synthetic aromatic substances or musk compounds ("white musk") are lipophilic contaminants, with the ability to accumulate in the components of the environment. They belong to the group of priority persistent pollutants and may occur in the components of the environment and human tissue. Since they have potential carcinogenic properties, efforts are currently being made to limit or ban their use in many regions worldwide.

Generally, synthetic aromatic substances or musk compounds can be divided into 4 groups: linear musk compounds, macrocyclic musk compounds, polycyclic musk compounds and nitro musk compounds. The last two groups are used most frequently as fragrances in personal hygiene products (perfumes, cosmetics, soaps, and shampoo), cleaning and disinfection products, industrial cleaning products, air fresheners, etc.

Synthetic aromatic substances were launched on the market in the early 20th century. The volume of their production has significantly increased over the last 50 years (Luckenbach & Epel, 2005). Galaxolide (HHCB) and Tonalide (AHTN) are examples of the most important musk compounds from the group of polycyclic compounds. Musk xylene, musk ketone and musk ambrette are well-known nitro musk compounds. The production of nitro musk compounds (i.e. compounds containing one or more nitro groups in a molecule) that are generally included in a group of substances posing a risk to the environment has decreased over the last years. By contrast, the production of polycyclic synthetic aromatic substances that are less toxic has increased and these substances are used in an abundant manner as additives in many products such as soaps, shampoos, deodorants, body lotions, perfumes, cleaning and disinfecting agents, air fresheners, industrial cleaning agents, etc. Unlike nitro

	Tonalide (AHTN, Fixolide)	Galaxolide (HHCB, Abbalide)	Musk ketone	Musk xylene
CAS	1506-02-1	1222-05-5	81-14-1	81-15-2
Physical state	Soluble in alcohol, oils, white solid crystal	Viscous liquid, No colour and stabile liquid	Soluble in alcohol, various oils, pale yellow crystals	Soluble in alcohol, oils, pale yellow crystals or fine crystalline powder
Molecular weight (g/mol)	258.41	258.39	294.27	297.27
Melting point (°C)	104.26	104.26	241.07	245.65
Boiling point (°C) at 25°C	325.92	325.92	560.94	570.75
Water solubility (mg l^{-1}) at 25°C	0.24	0.21	0.46	0.15
Henry's Law Constant ($atm.m^3.mol^{-1}$)	$2.28.10^{-4}$	$2.57.10^{-4}$	$1.33.10^{-19}$	$3.15.10^{-17}$
Log K_{OW}	5.70	5.90	4.30	4.90
Log K_{OC}	3.72	3.72	3.58	3.58

Table 2. Physico-chemical characteristics of musk compounds

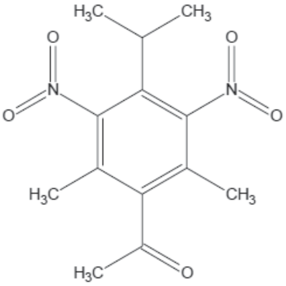
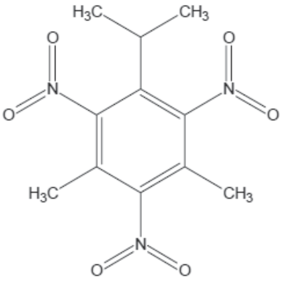
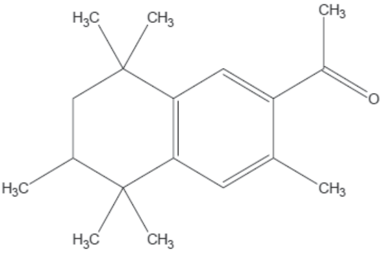
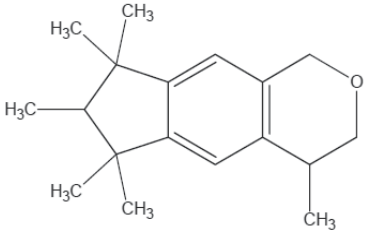
	
Musk ketone	*Musk xylene*
	
Tonalide	*Galaxolide*

Fig. 2. Structural formulae of selected musk compounds

musk compounds, polycyclic musk compounds are more resistant against light and bases, and bind to fabrics quite easily. They are therefore used in cosmetic products and detergents more frequently. Logically, the reduced production of nitro musk compounds resulted in the increased production of polycyclic substances.

Synthetic aromatic substances are lipophilic, persistent organic compounds that show bioaccumulation abilities in the aquatic ecosystem. They were detected in the samples of air and dust collected in self-contained premises (Regueiro et al., 2009). A number of studies revealed the presence of musk compounds in the tissues of aquatic animals and also in human tissues. Musk compounds were also detected in fat tissue, human milk and blood plasma (Lignell et al., 2008).

The recent studies provided the presence of musk compounds in abiotic and biotic matrices. After their application the AHTN and HHCB are generally transported to wastewater treatment plants via industrial and municipal wastewaters (Fromme et al. 2001; Valdersnes et al. 2006, Wombacher et al. 2009). The various concentrations of the polycyclic musks have been determined in sewage treatment plants (on influent and effluent), surface waters, sediment in varies region of Europe and their concentration in aquatic ecosystems is highly depended on the distance from wastewater treatment plants. These toxic compounds are now widely found in variety of biota in the many regions in the world and its typical organism (Tanabe 2005; Nakata et al. 2007). The potential impact of polycyclic musk compounds were studied in relation to biodegradation, bioconcentration, determination of their concentrations in environmental matrixes (Bester 2009; Onesios et al. 2009; Lee et al. 2010; Sumner et al. 2010).

2. Environmental analysis

The analysis of organic pollutants and the preparation of other samples required for the analysis start with the concentration of an analytes which can be achieved by using different methods, for example by liquid-liquid extraction, solid phase extraction, purge & trap, headspace or other techniques that may be time demanding and require sophisticated instruments and organic solvents.

The solid-phase microextraction method (SPME) is a simple and efficient sorption-desorption technique allowing the analytes to be concentrated. It is based on the exposure of a small amount of sorbent (extraction phase) to an excess of a sample. The goal of most SPME procedures used for sample preparation prior to analysis is to obtain a particular analytes in a sufficient detectable amount without any unwanted admixtures. In the case of SPME, analytes bind to a fibre until the steady state is reached. The SPME method is used in both qualitative and quantitative analysis. The accuracy and precision of the results is affected by a number of factors. The SPME method provides linear calibration dependence in a wide range of concentrations. The proper choice of fibre will ensure reproducible results even at low levels of analytes.

SPE is typically performed manually, but there are some significant disadvantages with this approach (Fatta et al., 2007):

- manual (off-line) SPE is time-consuming as well as labor-intensive and costly, which compromises productivity;
- exposure to hazardous or infectious matrices (such as sewage) involves safety issues; and,
- the recovery of the analytes can vary from batch to batch, causing reproducibility problems.
- direct injection of untreated samples;
- automatic sample clean-up and/or analyte enrichment;
- elimination of conventional manual sample pretreatment
- steps;
- faster procedures;
- methods are less prone to errors, resulting in Berger reproducibility;
- reduction of health risks; and,
- samples can be run unattended (e.g., overnight or over the weekend).

The methods of high performance liquid chromatography (HPLC) with diode-array detection (DAD) or mass detector (MS) are also the widely utilizing analytical method (HPLC-DAD, HPLC-MS) for determination of pharmaceuticals and musk compounds. The method gas chromatography with mass detector (GC-MS) or gas chromatography with atomic emission detector (GC-AED) detect compound with excellent sensitivity.

2.1 Sampling area

The first one is large-scale wastewater treatment plant of the City of Brno (catchment region for a population of 500,000 people). There is municipal waste water treated. This facility was launched in 1961 as classic two-stage plant with anaerobic sludge stabilization. In the period between 2001 – 2003 the overall reconstruction and extension of the WWTP was realized with the main objective to meet the treated wastewater effluent limits set by Czech and European standards and regulations, and to ensure sufficient capacity of the facility to accommodate the growing demand of the city of Brno with almost 500 thousand of inhabitants and several industrial facilities, but also of increasing number of the surrounding agglomerations successively connecting to the Brno sewerage system. Nowadays, the technology in WWTP Brno-Modřice corresponds to the EU parameters. The second WWTP collects waste water from laboratories and from research workplaces is there collected. The municipal WWTP uses technology of anaerobic stabilization of sewage sludges. This closing step is included to minimalize decomposition of residual organic material and mainly to eliminate pathogenic organisms. Three previous steps of water treatment include mechanical removing of settled elements, chemical treatment - defosfatation and denitrification in anaerobic tank. Follow biological treatment in activated tank, under oxygen conditions. Secondary settlement tank is final tank where is activated sewage sludge settled and separated out from treated water. The technology of small local WWTP located on the grounds of the University of Veterinary and Pharmaceutical Sciences in Brno involves aeration in activated tank, followed with separation of activated sewage sludges. Water is sanitized with chlorine and sewage sludges are thermic stabilised (125 °C/35min). Then water enters to communal sewerage system. The amber glass sample bottles were used for this collection. The samples were processed immediately or stored in a refrigerator till the following day. If necessary, the samples were filtered to remove rough impurities.

WWTP Brno-Modřice

WWTP at Veterinary and Pharmaceutical University Brno

Fig. 3. The sampling localities

Wastewater samples have been collected from the two wastewater treatment plant - municipal WWTP Brno-Modřice and WWTP at Veterinary and Pharmaceutical University Brno in one-day intervals and from in seven-days intervals. The sampling of waste water and sludge for experiments were from the WWTP inlet, outlet and lagoon (pond) from reason of comparing.

2.2 The analysis of pharmaceuticals

The application of advanced measurement technologies (e.g., gas chromatography with mass spectrometry (GC-MS) and GC with tandem MS (GC-MS2) or liquid chromatography with MS (LC-MS) and LC with tandem MS (LC-MS2)) to environmental analysis has allowed the determination of a broader range of compounds, including pharmaceuticals, and has therefore permitted more comprehensive assessment of environmental contaminants. LC-MS2 is becoming more commonly used in pharmaceuticals analysis because of its high sensitivity and its ability to confirm compounds (as compared with conventional LC with ultraviolet (UV) or fluorimetric detection). LC-MS2 allows separation and detection of compounds having the same molecular mass but different product ions, even if they co-elute. MS2 detection is therefore preferred for increased analytical sensitivity and selectivity in complex matrices, such as wastewaters. (Kot-Wasik et al., 2007).

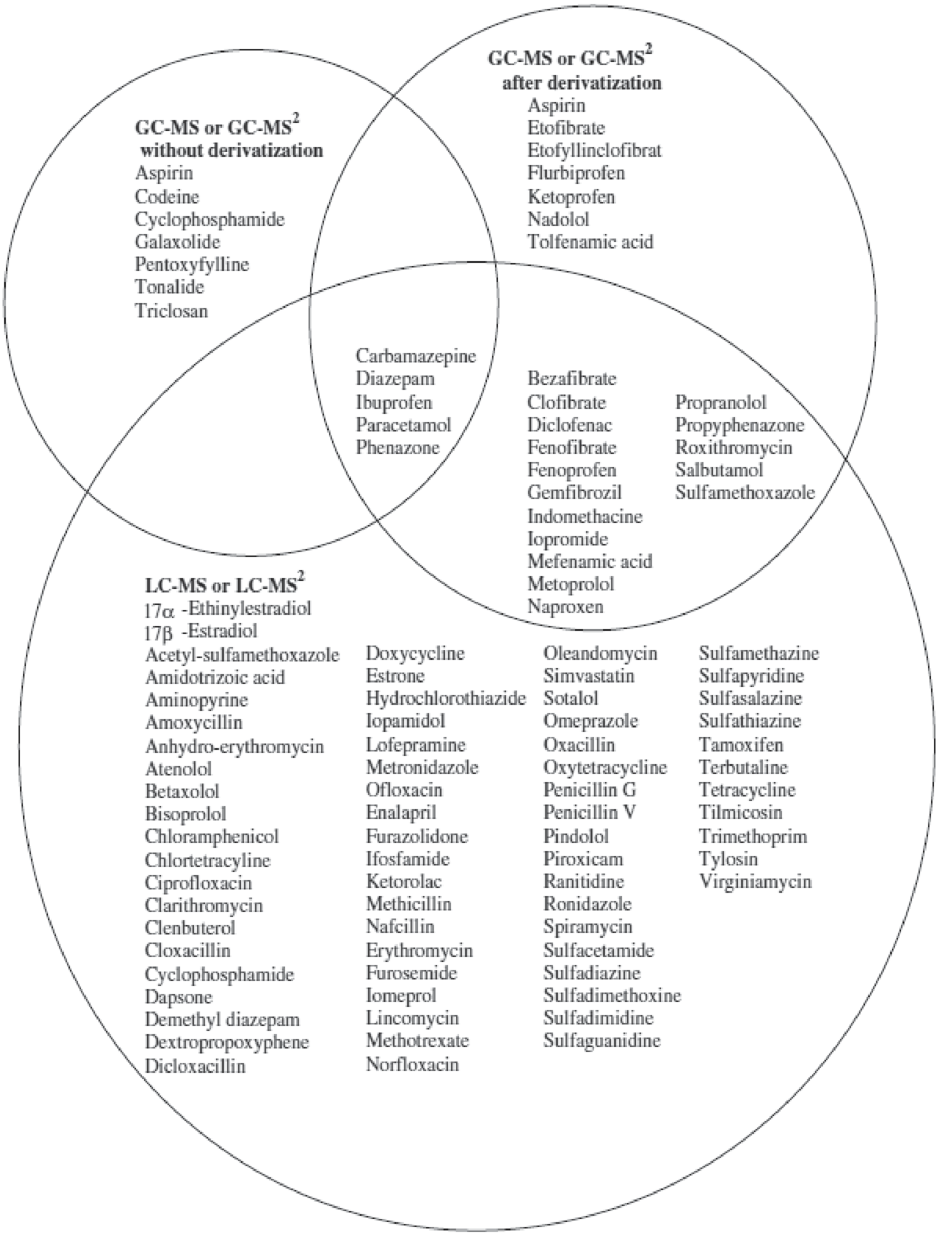


Fig. 4. Analytical methods applied for the most common pharmaceuticals in water and wastewater (Fatta et al.,2007)

Solid phase extraction (SPE) was employed as the separation method for isolation of given pharmaceuticals and high performance liquid chromatography (HPLC) with diode-array detection was used for their determination. The optimised and validation method was used to model samples of water. Obtained results were evaluated and their interpretation was done.

Several different columns were tested for diclofenac (Nonsteroidal anti-inflammatory substances – NSAIDs). Oasis HLB is column with hydrophilic-lipophilic balance. It includes copolymer divinylbenzen/N-vinylpyrrolidin. Often they are used for solid phase extraction of pharmaceuticals.

Sorbent with polymerization bonded oktadecyl phase is in ENVI-18. These columns are employed especially for environment. LC SAX was last tried column. It is ion exchanger column with polymerization bonded quaternary amine.

From the Table 3 it is implied, that the respective procedure for SPE is acceptable for diclofenac when using Oasis HLB and ENVI-18 cartridges. The Oasis HLB column was selected as the suitable one, due to highest yields of diclofenac from the cartridges tested. To find the optimal procedure SPE for both drugs, other SPE courses were tested on Oasis HLB columns.

SPE	Original diclofenac concentration (mg/ml)	Final concentration of diclofenac (mg/ml)	Diclofenac recovery %
Oasis HLB	0.0150	0.0042	28.04
ENVI-18	0.0150	0.0036	24.23
LC-SAX	0.0150	$2.37.10^{-5}$	0.16

Table 3. Recovery on SPE columns HLB when testing various procedures

The pharmaceutical Diclofenac and Ibuprofene were determined at inflow and outflow of WWTP Brno-Modřice. The determined concentration of ibuprophene at inflow was 1018.14 ng/l and at outflow 195.97 ng/l. The efficiency of water treatment process for Ibuprofene was 80%. The aim of this work was to develop both a suitable analytic method for analytes isolation from a given matrix and an optimal technique for identification and determination of analgetics residues in waters. Solid phase extraction (SPE) was employed as the separation method for isolation of given pharmaceuticals and high performance liquid chromatography (HPLC) with diode-array detection was used for their determination. The optimized method was used to model samples of water. Obtained results were evaluated and their interpretation was done

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Analytes	N	1	2	3	4	5
	Inflow					
Diclofenac	I1	NQ	NQ	NQ	NQ	NQ
	I2	1363.16	84.40	1874.95	2511.63	1970.58
	I3	1669.12	< LOD	2139.03	2439.98	2148.06
	Outflow					
	O1	NQ	NQ	NQ	NQ	NQ
	O2	5.69	NQ	NQ	NQ	NQ
	O3	9.29	NQ	NQ	NQ	NQ
Ibuprophene	Inflow					
	I1	NQ	NQ	503.78	2229.30	1170.20
	I2	NQ	NQ	272.50	1164.81	188.08
	I3	NQ	NQ	1271.06	193.30	432.21
	Outflow					
	O1	NQ	NQ	222.70	212.41	69.29
	O2	NQ	NQ	275.19	181.85	153.38
	O3	NQ	NQ	155.22	124.58	369.10

Table 4. The determined concentration of selected pharmaceuticals in waste water on inflow of WWTP Brno-Modřice (ng/l, n=10)

2.3 The analysis of musk compounds

In view of the physicochemical properties of musk compounds , most common analytical methods include separation by gas chromatography (GC) followed by online mass spectrometry (MS) Gas chromatography shows lowdetection limits, high selec tivity and sensitivity for determination of musk compounds.

Developments in the determination of synthetic musk fragrances in environmental matrices have been widely reviewed by A.M. Peck, 2006.

Traditional and worldwide used musk compounds Galaxolide®, Tonalide®, Musk xylene and Musk ketone were analysed. Samples were taken from the in influent, effluent and small pond in the WWTP Brno-Modřice municipal waste water treatment plant. Musk xylene was surprisingly found as the dominating compound, followed by Galaxolide, Tonalide and Musk ketone.

Head-space SPME with PDMS/DVB 65-μm fibre was used for the isolation of musk compounds from real waste water samples. Then the SPME was removed and sorbed compounds were immediately desorbed in the injector of GC/MS system. Mass spectrometer was operated in SIM mode list of target ions and qualifiers for traditional musks shows Table 1, those for Czech fragrances presents Table 2. External standard method and standard addition method were utilized for quantization; recovery of the whole analytical process was controlled by deuterated standards (Musk xylene D15, Tonalide D3).

During optimization, several real samples were also measured. It was found that the sample matrix had a significant negative effect on the determination of all analytes before ultrafiltration. HHCB and AHTN could only be detected in undiluted sample; however, after the standard addition, their response in the undiluted sample decreased. It was therefore decided to dilute samples at a ratio of 1:5 while maintaining the total volume of 14 ml. Distilled water was used for dilution. At this dilution and submersion, AMB and MK behaved as expected, showing the increasing response to the standard addition. In this case, it was decided to increase the dilution ratio to 1:10 and switch to the headspace. Thanks to the high concentrations of HHCB and AHTN in the undiluted sample, it was possible to employ greater dilution for the assay of HHCB and AHTN in the real sample.

Samples after ultrafiltration were not as difficult to analyse as samples before ultrafiltration.

Sample	Galaxolid (HHCB)		Tonalid (AHTN)		Musk ketone (MK)	
	Inflow (ng/ml)	Outflow (ng/ml)	Inflow (ng/ml)	Outflow (ng/ml)	Inflow (ng/ml)	Outflow (ng/ml)
1	187.7	0.66	24.64	0.179	2.28	0.082
2	575.1	NQ	141.40	0.220	0.74	0.010
3	54.1	1.84	33.32	0.695	2.56	0.020
4	213.3	0.56	33.69	0.231	ND	ND
5	31.1	0.55	12.80	0.811	ND	ND
6	210.8	1.74	15.86	0.736	ND	ND
7	86.7	0.67	5.19	0.172	ND	NQ
8	50.1	0.50	10.62	0.156	ND	ND
9	772.0	0.57	57.56	0.145	ND	NQ
10	77.6	0.52	12.31	0.151	ND	NQ
Mean	225.9	0.85	34.74	0.350	1.860	0.037

Table 5. The levels of analytes detected in samples,
(inflow = before ultrafiltration, outflow = after ultrafiltration (n=10))

As seen in Table 5 the highest levels were observed for HHCB in samples before ultrafiltration where the maximum level was 772.0 ng/ml whereas the lowest level was 31.1 ng/ml. The levels in samples collected at the outflow were low. Maximum concentrations were observed in samples before ultrafiltration where the maximum estimated concentration was 141.40 ng/ml. The minimum concentration in samples before ultrafiltration was 5.19 ng/ml. The levels of AHTN in samples after ultrafiltration varied in a range of 0.811-0.145 ng/ml.

Musk ketone (MK) was found in the inflow only during first three days of analysis. Its respective levels varied in a range of 0.74-2.062 ng/ml. The performance of elimination of three monitored analytes reached 99%. The performance for HHCB varied in a range of 96.60-99.93%, for AHTN in a range of 93.66-99.84%, and for MK in a range of 96.43-99.23%.

The flow of wastewater in the ultrafiltration step varied in a range of 160-300 l/h. The facility is operated 10 hrs a day on working days. The annual balance is based on 253 days. The mean flow of wastewater through the ultrafiltration step is approximately 230 l/h. The discharge into the municipal sewer system is important, i.e. water that flows to the wastewater treatment plant. For better clarity, the amounts of analytes that enter the ultrafiltration step are also provided. All values are recalculated to the mean flow of 230 l/h.

Musk compounds are widely used as fragrances in cosmetic industry and its related fields. Due to their massive use over the last few decades, they are currently being detected in the majority of components of the environment. The first step to assay musk substances is to optimize the procedures of the isolation, identification, and quantification of individual

N	Compound concentration (ng.l^{-1})											
	Galaxolide (HHCB)			Tonalide (AHTN)			Musk xylene (MX)			Musk ketone (MK)		
	Inflow	Outflow	Pond	Inflow	Outflow	Pond	Inflow	Outflow	Pond	Inflow	Outflow	Pond
1	921	530	71	262	80	37	1223	955	972	56	47	71
2	996	504	107	248	120	47	961	697	592	57	33	34
3	762	646	111	194	180	56	1019	688	543	60	54	34
4	739	528	101	192	132	56	1100	558	417	56	49	49
5	880	858	149	217	170	66	1135	1491	686	58	54	27

Table 6. Concentrations of traditional musks in Brno-Modřice WWTP (n=10)

analytes investigated. Great efforts during method optimization have been made to develop an efficient method that would be not time-consuming; eliminate possible errors during the entire procedure; and prevent the pollution of the external environment. Samples of wastewater collected at a cosmetic company before and after the ultrafiltration step were analysed using the SPME method (solid phase microextraction), followed by GC/MS (gas chromatography combined with mass spectrometry) as an end-point. The results are summarized in the following points:

- Analytes were determined in real samples using the SPME method and GC/MS as an analytical end-point. All samples collected before ultrafiltration contained HHCB and AHTN. Concentrations of HHCB and AHTN varied in a range of 31.1-772 ng/ml and 5.2-141.4 ng/ml, respectively. The levels of musk ketone in wastewater flowing to the ultrafiltration step varied in a range of 0.74-2.56 ng/ml
- All samples of wastewater flowing from the ultrafiltration step contained AHTN at levels that ranged from 0.15 to 0.81 ng/ml. HHCB was detected in almost all samples of wastewater (except for one) collected after the ultrafiltration step. The levels of HHCB varied in a range of 0.52-1.84 ng/ml. MK was found in wastewater before the ultrafiltration step only 3times. Its levels varied in a range of 0.01-0.08 ng/ml.
- The calculation assumes 253 working days in 2010 to estimate the amount of discharged substances per year, with 20 days for every month. The amount of analytes is as follows: HHCB - 131.42 kg per year, 10.39 kg per month; AHTN - 20.21 kg per year, 1.60 kg per month; MK - 1.08 kg per year, 0.09 kg per month.
- Quantities of individual monitored musk compounds discharged were assessed in the same manner as in the previous point. The amount of discharged analytes is as follows: HHCB - 1.04 kg per year, 0.08 kg per month; AHTN - 0.20 kg per year, 0.02 kg per month; MK - 0.02 kg per year and 1.70 g per month. The efficiency of ultrafiltration employed to remove musk substances ranged from 96 to 99% in this particular case.

Although the efficiency of ultrafiltration is high, the respective analytes are still present in the wastewater that flows to the wastewater treatment plant.

3. Environmental toxicology

Environmental toxicology or ecotoxicology deals with potentially harmful effects on organisms of countless man-made chemicals and wastes released into biosphere Ecotoxicity tests, biotests, bioassays are the only way how to find out experimentally ecotoxicological characteristic of chemicals, hazardous wastes and other various matrices. Bioassays could give answer on following questions:

- interaction between the substances (additive, synergistic, antagonistic etc)
- toxic effect after degradation and transformation of products
- quantity of the bioavailable chemicals
- effects on biota.

Testing system is exposed to various concentration of tested substance at exactly defined conditions (Park, et al., 2005). Contrary of the chemical analysis the tests are not able to identify the xenobiotics in the environment, on the other hand their advantage consist in prediction of effect on testing biological material. Advantages of biotests are also unquestionable. There are able to characterize hazard of xenobiotics in environment contrary to chemical analysis, which is able xenobiotics only identify and quantify. In addition in ecosystem hazardous compounds no exist separately but in various combinations wit mixture toxicity and environmental impact (Hoffmann, 2003). Biotest could be classified from various points of view. Standard and alternative test are the most basic classification. Standard toxicity tests are recommended by different international organizations such as ISO, EPA, OECD. The necessity to screen many samples for toxicity have lead to the increasing application of rapid miniaturized toxicity tests that are also called microbiotests, alternative assays, small-scale tests, microtests or second generation biotests. A microbiotest or alternative assay can be defined as involving the exposure of a unicellular or small multicellular organism to a liquid sample in order to measure a specific effect. Alternative toxicity tests have attractive features in comparison to standard toxicity tests; high cost efficiency, modest time and laboratory space requirements, possibility to test many samples at once, low sample volume requirements, (Blaise, 1991; Rojíčková, 1998). According to exposure time are the acute, semi-chronic and chronic tests used. The tests could be classified as terrestrial or aquatic. The aquatic tests use water solution of tested compounds or water leaches of tested matrices. In the terrestrial tests are testing organisms in direct contact with tested matrices. The selection of biotests used for toxicity assessment depends on the type of information that is required, the state, physical and chemical features of a sample or substance investigated and on sensitivity and relevance of a test species. However, if only one single test is used, the estimated toxicity would reflect the sensitivity of that test only, with a risk for underestimation of the potential toxicity for that particular substance with regard to the ecosystem. The use of a battery of biotests, representing differences in sensitivity of test organisms from various trophic levels is recommended to prevent this situation. Therefore it is important to identify potential toxins and understand the magnitude of their impact on different terrestrial and aquatic ecosystems (Schowanek et al., 2004)

3.1 Procedure of ecotoxicity test

The aim of the ecotoxicological tests is the determination of effective concentration (EC), eventually lethal concentration (LC) or inhibition concentratin (IC) to assess the effect of chemical compound, water wastes and leaches of various materials (sediments, soils, wastes etc.) on aquatic and terrestrial organisms. From this date the ecotoxicological impact to aquatic and terrestrial ecosystems can be also derived. These concentrations of tested compounds cause the mortality of 50 % testing organisms or 50% inhibition growth rate in relation to control tests. Lower values of LC(EC,IC)50 indicate higher toxicity to the organisms. In accordance with testing regulation the limit test, preliminary tests and definitive test were conducted with single compounds. In limit test concentration 100 mg L^{-1}

of tested compounds is used. Preliminary tests served to find approximate toxicity of the chemical compounds if is not known. In this case (range finding test) the dilution series was following 100 mg L^{-1}, 10 mg L^{-1}, 1 mg L^{-1}, 0,1 mg L^{-1} and 0,01. The definitive test served to calculation value of LC(EC,IC)50. Range of dilution series of the definitive test was determined by results of preliminary test.

From obtained experimental ecotoxicological endpoints (EC50, IC50, LC50) of ecotoxicity tests the quantification of ecotoxicity values is calculated. These parameters refer to the concentration of toxic substance that results in 50% reduction of end-point relative to control at a given period of time (ISO/TS 20281:2006). The results of tests are suitable statistical evaluated with using the statistical software. The significant differences ($p<0.05$) in the measured parameters in each of tests (inhibition of growth, immobilization etc.) are evaluated by ANOVA test.

	
Daphnia magna	*Thamnocephalus platyurus*
	
Lemna minor	*Sinapis alba*

Fig. 5 The testing organisms

3.1.1 *Daphnia magna* acute immobilization test

The testing organisms *Daphnia magna* is a component of freshwater zooplankton. It refers to group *Arthropoda, Branchiopoda, Daphnidae*. Daphnia are small arthropods of 1–5 mm in size. They live in various aquatic environments ranging from acidic swamps to freshwater lakes, ponds, streams and rivers. Often they are found in large amounts. Ontogenesis of individual is direct without larval stages. During the year there is one or several biological cycles in which parthenogenetic generations are alternated by bisexual generations which enclose the cycle. This test serves for testing of effect of chemicals on plankton organisms. The importance of the test consists in plankton organisms served as feed for fish and are therefore significant component of water biocoenosis and food chain. For the calculation value of EC50 alternative Daphtoxkit F^{TM} was used (purchased from MicroBioTests Inc., Gent, Belgium). This toxkit includes dormant eggs in chitinous capsule (ephippia) of crustaceans *Daphnia magna.* The Daphtoxkit F^{TM} makes use of such dormant eggs produced under controlled conditions with a standard Daphnia magna strain. The tests were conducted following the *Standard Operational Procedure* of the Daphtoxkit F™ which is in accordance with the OECD and ISO test protocols for the acute *Daphnia magna* toxicity tests (OECD, 2004; ISO, 1996). Standard Freshwater was prepared with the concentrated salt solutions included in the kit. This medium, which has the composition recommended by the ISO for acute toxicity tests with *Daphnia magna,* is used as a hatching medium and as a dilution medium for the preparation of the toxicant dilution series. The assays were carried out in the multiwell test plates provided in the kits. Ephippia were hatched in petri disches with Standard Freshwater (ISO) medium three days before test at temperature 20-22 °C, continuous illumination of 6 000 lux. Two hours prior to test were the neonates pre-feeded wit suspension of spirulina powder to proved them energetic reserve. Daphnids aged less than 24 were exposed to dilution series of tested compounds. 20 individuals were subdivided in four replicates for every concentration of multiwell plate (well volume is 5 ml). The some procedure was done with control Daphnids (20 individual subdivided in 4 wells). In fine was multiwell plate sealed with a strip of parafilm and covered with lid. Experiments were conducted at temperature 20 °C in darkness incubator. Immobility (the endpoint for effect calculation) was obserwed after 24 and 48 h. The values of 24hEC50 and 48hEC50 were calculated by probit analysis. The test was considered valid if the number of dead organisms in the control did not exceed 10%.

3.1.2 *Thamnocephalus platyurus* acute immobilization test

For calculation value of LC50 alternative test Thamnotoxkit F™ was used (purchased from MicroBioTests Inc., Gent, Belgium). This test is often use to screening toxicity in freshwater and determination of acute toxicity Thamnotoxkit is similar to Daphtoxkit F™ contains all the materials to perform six complete acute (24-h) toxicity tests (range-finding or definitive) based on mortality as the effect criterion the assays were performed on larvae of the fairy shrimp *Thamnocephalus platyurus* hatched from cysts. The procedure of this test is similar as mentioned above. Standard freshwater was prepared by diluting the concentrated salt solutions included in the kit to obtain 1 l of medium (moderately hard reconstituted freshwater made up according to the US EPA formula) which serve for hatching the cysts and to prepare the toxicant dilution series. The eggs of crustaceans were hatched 24 hours before testing at temperature 25 °C under continuous illumination 4000 lux. Design of the test is similar to Daphtoxkit F™ test, multiwell plates are smaller (1 ml volume), and number of testing organisms in each well is 10. Each toxicant concentration was tested out in three replicates. The test procedure followed the *Standard Operational Procedure* of the Thamnotoxkit FT™ magna microbiotest. The test plates subsequently were incubated at 25 °C in darkness. Lethality (endpoint for effect calculation) was observed after 24 h. The values of 24hLC50 were calculated by probit analysis. The test was considered valid if the number of dead organisms in the control did not exceed 10%.

3.1.3 Root growth inhibition toxicity test on *Sinapis alba*

The test was developed for testing of harmlessness of waste waters, which could be used for sewage irrigation. Effect on seed germination and root growth of mustard (*Sinapis alba*) in first stadium of development is tested. Test consists of cultivation of the seeds on beds saturated with solutions of tested chemical in comparison with seeds grown on bed saturated with dilution medium. Mustard, *Sinapis alba* L., (*Brassica alba* L.) belongs to *Brassicaceae*. It is annual, early spring grown oil bearing plant. Mustard root is thin; shoot is erect, hairy and up to 150 cm high with clear green leaves. Seeds are relatively big, in comparison with other *Brassicaceae* and are yellow or whiteyellow of a round shape. They reach to 1.5 - 4 mm in diameter. After germination, simple root with hypocotyle grows up.

The high quality seeds of *Sinapis alba* are exposed to solution of tested compounds at temperature 20±2 °C in the darkness incubator for 72 hours. The seeds (30 pieces) for every tested concentration are placed on filter paper in Petri-dishes. Paper is moistened with solution of tested compounds. Dilution series of tested compounds were prepared by dilution of stock solution of tested compounds in diluent medium. Diluent medium was prepared by filling up of 2.5 ml from stock solution of every salt to 1 l volume flask. Stock solution of $CaCl_2$. $2H_2O$ was prepared by solution of 11.76g $CaCl_2$. $2H_2O$ in 1l volume flask. The other stock solution was prepared by solution of 4,93 g of each salt ($MgSO_4 . 7H_2O$, Na HCO_3, KCl) in 1l volume flask. Two replicates were done for every dilution series. For calculation values of IC50 the lengths of hypocotyls of germinated seeds in tested and in control group were measured. The inhibition of root growth (the endpoint for effect calculation) was measured after 72 hours. The test was considered valid if the number of germinated seeds in control was at least 90 %; organisms in the control did not exceed 10%.

3.1.4 *Lemna* growth inhibition test

The test has been used for toxicity of solutions and suspensions testing. A higher freshwater plant, duckweed (*Lemna minor* L.) is used. From this point of view we can talk about semi-chronic exposition, where immediate effect, as well as long-term effect, is involved and visible in growth of new generation of plants.

Taxonomically *Lemna minor* L. belongs to angiosperms (*Angiospermophyta*) monocotyledonous plants (*Monocotyledonopsida*), *Lemnaceae*. These macrophytes take place at maintained water areas where they serve as feed e.g. for fish and water birds. *Lemna minor* (duckweed) cover surface of stagnant waters and are the most known species from pleustonic communities.

Lemna tests with duckweed *Lemna minor* were performed according OECD Test No. 221: Lemna sp. Growth Inhabition Test using Steinberg medium (OECD 2006). Biotest were carried out in 200 ml beakers filled with 150 ml solution (dilution series of tested compounds diluted in Steinberg medium). The beakers were inoculed with 14 fronds. Plants with two or three fronds were chosen as inoculum. Six control and treatment replicates were used. Test were carried out at temperature 24 ± 2°C, light intensity was adjusted at 8000 lux. Test duration was 7 days (168 h). Number of fronds was controlled at days 0, 3, 5 and 7. The second monitored characteristic was the dry mass determinate at temperature 60 °C to constant weight. Dry mass was determined on the beginning of the test too. For this purposes were 6 additional control inoculated. Growth inhibition (measured as the increase in number of fronds during 7 days of incubation as compared to a corresponding control) was recorded after 168 h. Growth inhibition as the toxicological endpoint served for calculation value of 168hIC50. The test was considered valid if the number of fronds grown eightfold.

3.2 The ecotoxicity of chemicals

Synthetic musk compounds, pharmaceutical residues (particularly analgesics and antibiotics) were ecotoxically evaluated. Ecotoxicity was assessed by alternative tests using species such as *Thamnocephalus platyurus* and *Daphnia magma* and a phytotest using white mustard (*Sinapis alba*) as a terrestrial testing organism and *Lemna minor* as water testing organisms. The mentioned species were used to assess the effect of musk compounds and pharmaceuticals on the aquatic ecosystem (*Lemna minor, Thamnocephalus platyurus, Daphnia magna*) and on terrestrial plants represented *Sinapis alba*. Test species mentioned above were also used to assess the ecotoxicity of sludge originating from a particular wastewater treatment plant, at some stages of sludge treatment.

3.2.1 Ecotoxicological evaluation of pharmaceuticals

Pharmaceuticals are environmentally were similar to other chemicals. In fact, high quantities of pharmaceuticals are discharged into sewage treatment plants. Local discharge of pharmaceuticals also contributes to environmental contamination due to high concentrations in small sites. The ecotoxicological effects of drugs on different levels of the biological hierarchy, from bacteria to the entire biosphere, are not well known. They are biologically active compounds that may interfere with specific biological systems (e.g. enzymes) or generically act depending on their properties. (Isidori et al. 2005). The growing use of direct toxicity assessment is a result of existing or new regulation implementing (e.g. EU Directive 93/67/EEC, REACH). International and national authorities have available ecotoxicity biotests which represent useful tools for the prediction of environmental impacts. EU Directive 93/67/EEC (Commission of the European Communities, 1996) classifies substances to their EC50 values in different classes; < 1 mg L^{-1} , (very toxic to aquatic organisms); 1-10 1 mg L^{-1} (toxic to aquatic organisms); 10-100 mg L^{-1} (harmful to aquatic organisms) substance with value EC50 above 100 mg L^{-1} would not be classified. Ibuprofen and diclofenac belong to the group of the nonsteroidal anti-inflamatory drugs. This one are the most frequently identified in detectable concentration in environment and in sewage water. The concentrations were between 0.01-510 µg L^{-1} for diclofenac and 0.49-990 µg L^{-1} for ibuprofen. Elimination of these pharmaceuticals in WWTP is something about 87 % for ibuprofen and 49-59% for diclofenac (Heberer, 2002; Kümmerer, 2002; Kosjek et al., 2007). Cleuvers (Cleuvers, 2003) summarized results of some studies. The following concentration are reported; diclofenac ≤ 1.59 µg L^{-1}, ibuprofen ≤ 3.35 µg L^{-1}, acetylsalicylic acid (ASA) 1.51 µg L^{-1} in sewage, lower concentration (0.01-0.5 1 µg L^{-1}) in river water, Ternes (Ternes et al., 1998) reported concentration of above mentioned pharmaceuticals and of naproxen some > 1 µg L^{-1} in WWTP and again lower concentration in surface water. Data summarized by Watkinson (Watkinson, et al. 2007) indicate that WWTPs often partially remove selected drug 20-90 %. They could be present in effluents and consequently in surface water. Isidori (Isidori et al., 2005) reported results from studies concerning occurrence antibiotics in water; lincomycin, erythromycin and clarithromycin in the rivers Po and Lambro in Northern Italy were detected at concentrations between 10 and 100 ng/L, in Switzerland, quinolones occurred at effluents at concentrations between 249 and 45 ng/L, respectively. Reported concentration are not extremely high contrary others pollutans, but drugs should stay in the centre of researches, because of their biological activity.

Ecotoxicological evaluation of some pharmaceuticals were conducted: from the group of non-steroidal anti-inflammatory substances Diclofenac sodium (2-[(2,6-dichlorophenyl)amino]benzeneacetic acid, Ibuprophen sodium 2-[4-(2-methylpropyl)phenyl]propanoic acid, Ampicillin from the group of antibiotics. (2S,5R,6R)-6-[[(2R)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid and Penicillin G 2S,5R,6R)-3,3-dimethyl-7-oxo-6-[(2-phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. Some studies were conducted to calculate ecotoxicological values for drugs. For diclofenac values of 30minEC50 on *Vibrio fischeri* for were 11.45 mg L^{-1} for *Cerodaphnia dubia* value of 48hEC50 22.70 mg L^{-1} for *Daphnia* *magna* 68 mg L^{-1} and for *Lemna minor* EC50 7.50 mg L^{-1}. For ibuprofen value of EC50 101.20 mg L^{-1} on *Daphnia magna* 342 mg L^{-1} in acute algal test on *Desmodesmus subspicatus*, 173 mg L^{-1} in acute toxicity test on fish and 22.00 mg L^{-1} is value of EC50 for Lemna minor (Cleuvers, 2003; Ferrari et al., 2003; Ferrari et al., 2004; Jemba 2006).

Informations concerning ecotoxicity of penicillin G and ampicillin on above mentioned organism are sporadic. Avaiable data served for preparing dilution series in preliminary tests. On the ground results of preliminary test were definitive test conducted. Achieved results summarized table 7.

Substances	*Daphnia magna*		*Thamnocephalus platyurus*	*Sinapis alba*	*Lemna minor*
	24hEC50 (mg.L^{-1})	48h EC50 (mg.L^{-1})	168hIC50 (mg.L^{-1})	72hIC50 (mg.L^{-1})	24hLC50 (mg.L^{-1})
Diclofenac-Na	53.0 (48.6 – 56,1)	17.2 (15.8 – 19.1)	169.4 (162.2 - 174.1)	83.8 (77.6 – 85.4)	15.2 (13.6 – 16.2)
Ibuprophen-Na	106.4 (96.4 – 110.0)	56.4 (53.7 – 59.6)	195.9 (188.7 – 197.0)	122.2 (118.6 – 125.4)	200.8 (196.4 – 205.0)
Penicillin-G	874.4 (867.0 – 879.5)	878.5 (871.8 – 883.2)	n.d.	653.4 (647.1 – 655.6)	857.2 (854.3 – 860.3)
Ampicillin	823.2 (815.0 – 831.1)	850.5 (839.8 – 858.4)	n.d.	286.7 (281.0 – 291.2)	650.3 (646.7 – 651.5)

Table 7. The ecotoxicity endpoints to crustaceans and plant testing organisms for pharmaceuticals

In most of biotests diclofenac exhibits greatest ecotoxicity, follows ibuprofen, ampicillin and penicillin G. It corresponds with results presented by Wollenberg (Wollenberg et al., 2000). Ecotoxicological values for some antibiotics were approximately 1000 mg.L^{-1} oxytetracycline, 680 mg.L^{-1} tylosine. It seems that antibiotics of penicillane (penicillin, ampicilin) and tetracycline (oxytetracycline) exhibit only low acute toxicity. According EU Directive 93/67/EEC belongs to the group of chemicals which would not be classified. Ibuprofen and diclofenac on the basic of scheme of classification would be classified as potentially harmful to aquatic organisms. In spite of higher ecotoxicity of NSAIDs acute toxicity is unlikely. With regard to purpose for which pharmaceuticals are generated (bring some benefit to alive organisms) strong acute effects caused by specific mechanisms may actually not be expected. In addition value of EC50 for *Daphnia magna* is manifold higher than measured in environment. From this point of view is prediction of chronic effect much more relevant. Moreover residues of pharmaceuticals do not exist by itself in the environment. Toxicity of a single substance could increase strongly in combination with other especially when mode of action is similar. On the basis of these facts is necessary to test toxicity of mixture compounds on battery of organisms representing various levels of ecosystem (Cleuvers 2003 & Fatta-Kassinos, 2010).

3.2.2 Ecotoxicological evaluation of musk compounds

Polycyclic musks, the common name for synthetic musks with rings in their chemical structure, are the most commonly produced and used musks. They include substances such as traseolide (ATII), celestolide (ADBI), fixolide/tonalide (AHTN), versalide (ATTN), galaxolide (HHCB), etc. *Nitromusks*, the common name for a group of (artificial) nitrogen-containing musks (produced by nitration of organic compounds), includes a number of compounds, such as: musk ketone, musk ambrette, musk tibetene, musk alpha and musk moskene (in addition to musk xylene) (; Balk and Ford, 1999; HERA, 2004).

The musk tested compounds were Galaxolide, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[*g*]-2-benzopyrane, Tonalide 1-(5,6,7,8-tetryhydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethynone, Musk-ketone 1-tert-butyl-3,5-dimethyl-2,6-dinitro-4-acetylbenzene and Musk-xylene 1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzene.

The effect of musk compounds on the organism were studied from several view namely for one organism as the acute or subchronic toxicity (Boleas et al. 1996; Carlsson & Norrgren 2003; Dietrich & Hitzfeld 2004; Mori et al. 2006). The acute toxicity of AHTN and HHCB were tested on the algae (*Pseudokirchineriella subscapitata*), crustacean (*Daphnia magna*), springtails (*Folsomia candida*), nematode worm (*Caenorhabditis elegans*), earthworms (*Eisenia fetida*), rainbow trout (*Oncorhynchus mykiss*), zebrafish (*Danio rerio*), brook minnow (*Pimephales promelas*), South African frog (*Xenopus laevis*) and bluegill sunfish (*Lepomis macrochirus*).

The table 8. shows the ecotoxicological obtained dates from using tests for tonalide, galaxolide, musk ketone and musk xylene.

Substances	*Daphnia magna*		*Thamnocephalus platyurus*	*Sinapis alba*	*Lemna minor*
	24hEC50 (mg.L^{-1})	48h EC50 (mg.L^{-1})	168hIC50 (mg.L^{-1})	72hIC50 (mg.L^{-1})	24hLC50 (mg.L^{-1})
Tonalide (AHTN)	1.51 (1.48 – 1.53)	1.33 (1.29 – 1.36)	1.58 (1.55 – 1.60)	5.42 (5.38 – 5.45)	5.20 (5.18 – 5.22)
Galaxolide (HHCB)	1.22 (1.19 – 1.24)	1.12 (1.08 – 1.13)	1.14 (1.11 – 1.16)	4.92 (4.87 – 4.95)	4.62 (4.58 – 4.66)
Musk ketone	2.33 (2.28 – 2.35)	2.13 (2.10 – 2.15)	6.14 (6.12 – 6.17)	4.84 (4.79 – 4. 87)	5.36 (5.32 – 5.40)
Musk xylene	2.39 (2.32 – 2.41)	2.22 (2.18 – 2.26)	6.16 (6.13 – 6.20)	5.68 (5.65 – 5.71)	5.36 (5.33 – 5.39)

Table 8. The ecotoxicity endpoints to crustaceans and plant testing organisms for polycyclic musks and nitomusks

The higher ecotoxicity is typical for polycyclic musk compounds (AHTN and HHCB), but results showed the lower ecotoxicity for nitromusk compounds (musk ketone and musk xylene). The sensitivity of organisms is various. The fresh crustaceans (*Daphnia magna* and *Thamnocephalus platyurus*) and *Lemna minor* are most sensitive than terrestrial plant (*Sinapis alba*).

3.3 Ecotoxicological evaluation of the sludges from wastewater treatment plant (WWTP)

Modern sanitary practices result in large volume of human waste, as well as domestic and industrial sewage, which are collected and treated at common collection points WWTP. The growing use of sewage sludge as fertilizer results in many studies concerning their chemical analysis and hazard assessment (Jones-Lepp and Stevens, 2003; Fatta-Kassinos et al., 2010). Wastewater undergo preliminary, primary, secondary and in same cases tertiary treatment before sewage sludge are produced. Wastewater treatment unit operations and processes have three important parts. Physical unit operations - screening, comminution, flow equalization, sedimentation, flotation, granular-medium filtration, Chemical unit operations – chemical precipitation, adsorption, disinfection, dechlorination, other chemical applications, Biological unit operations - activated sludge process, aerated lagoon, trickling filters, rotating biological contactors, pond stabilization, anaerobic digestion, biological nutrient removal. Sludge resulting from wastewater treatment operations is treated by various methods in order to reduce its water and organic content and make it suitable for final disposal and reuse. Anaerobic wastewater treatment is the biological treatment of wastewater without the use of air or elemental oxygen. Anaerobic digestion/stabilization reduces the volatile solid content by approx. 60 to 65 %, and significantly reduces pathogens. The sludges from waste water treatment are several types and its composition and properties depend on the level of the waste treatment.

- Raw sludge is untreated non-stabilized sludge. It tends to acidify digestion and produces odours.
- Primary sludge is produced through the mechanical wastewater treatment process. The sludge amassing at the bottom of the primary sedimentation basin is also called primary sludge. Primary sludge consists to a high portion of organic matters, as faeces, vegetables, fruits, textiles, paper etc.
- Activated Sludge - The removal of dissolved organic matter and nutrients from the wastewater takes place in the biological treatment step. It is done by the interaction of different types of bacteria and microorganisms. The resulting sludge from this process is called activated sludge. The activated sludge exists normally in the form of flakes, which besides living and dead biomass contain adsorbed, stored, as well as organic and mineral parts.
- Return activated sludge - The activated sludge flows from the biological aeration basin into the final clarifier. The main part of the separated sludge, which is transported back to the aeration basin, is called return activated sludge.
- Secondary sludge (Excess sludge) - To reach a constant sludge age the unused biomass has to be removed from the biological treatment system as excess sludge. The excess sludge contains not-hydrolysable particulate materials and biomass due to metabolisms.
- Tertiary sludge - Tertiary sludge is produced through further wastewater treatment steps e.g. by adding a flocculation agent.

The sludges from WWTP are various applications, mainly in agricultures and recultivation in relation to environmental Directive 86/278/EEC (Council directive, 1986). On the other hand they could represent big problem because of concentrated xenobiotics. The heavy metals Zn, Cu, Co, Pb, Hg, Cr, Cd, anthropogenic xenobiotics (PCB, dioxins, PAHs, etc) are serious contaminants of sludges. The stabilized sludges with containing organic matter, nutrients and biologically active substances represent the source of failure nutrients and elements (N, P, K, Ca, Mg) and also organic matter, but their application on the land is limited by xenobiotics and pathogen organisms. In 2006, were produced in Czech republic 220700 tons of sewage sludge (expressed in dry matter) 75 % of sewage sludge was land-applied, 0,9 % incinerated, 13 used in other methods and 13 % were disposed on dumps. Ratio of disposed sewage is relatively high. Some studies indicate that not only traditional analytes [i.e., PAHs, PCBs, polychlorinated naphthalenes (PCNs -structurally similar to PCBs, several of which exhibit dioxin- like toxicity), polychlorinated n-alkanes (PCAs)], and for a class of PPCPs - synthetic musks are present at significant concentrations (Jones-Lepp and Stevens, 2003). The Hazardous Waste Council Directive 91/689/EEC set the rules for the management, recovery and correct disposal of hazardous wastes. The directive has established, in its Annex I, different categories of wastes In order to characterise wastes as hazardous, must display any of the 14 properties specified in Anne III. Property labelled H14 – "ecotoxic" exhibits substances and matrices which present or may present immediate or delayed risk for one or more sectors of the environment (Pablos et al., 2009). To decide if wastes are hazardous ecotoxicological values LC(EC, IC)50 resulting from bioassay provided by legislation on *Daphnia magna, Sinapis alba,* fresch water algal *Scenedesmus subspicatus and* vertebrate *Danio rerio* are required.

Sludge mainly collected from wastewater treatment plants (WWTP Brno-Modřice) were subjected to ecotoxicological characterization to provide a preliminary assessment of their ecotoxicity. The various type of sludges were analyzed – anaerobic stabilized sewage sludge (AS), dewatered anaerobic stabilized sewage sludges (DWAS) and desiccated stabilized sewage sludge (DSAS) and activated sludge (ASV) from the small WWTP of the University of Veterinary and Pharmaceutical Sciences in Brno were tested. Several toxicity tests were performed under standard laboratory conditions using freshwater crustaceans (*Daphnia magna*, *Thamnocephalus playturus*) and aquatic and terrestrial plants *Sinapis alba*. The values of 24hLC50, 48hEC50 and 72hIC50 are the basic data for the ecotoxicological assessment of the sludge and for their classification following the Czech legislation. Following legislation concerning ecotoxicological evaluation of waste were the test conducted on water leaches of sewage. Wastes are extracted with the corresponding test medium in ratio 10L/kg dry water for 24h. Leaches were diluted using dilution medium corresponding to each testing organism in dilution series similarly to procedure with chemical substances (50, 100, 300, 500, 700 ml L^{-1} and leach non-diluted only saturated with salt belonging to testing organisms – I series). To compare ecotoxicity of sewage of various humidity, were sludge diluted with water to have uniform dry matter as the most humid sewage (AS – II series. In case that

	Daphnia 48hEC50 (ml L^{-1})		Thamnocephalus 24hLC50 (ml L^{-1})		Sinapis 72hIC50 (ml L^{-1})	
	I series	II series	I series	II series	I series	II series
AS	52.04	-	22.81	-	203.62	-
DWAS	136.38	340.37	39.57	128.02	262.00	-
DSAS	236.42	540.21	139.64	343.15	266.56	-
ASV	38.17	426.89	129.57	422.34	-	-

- value of IC50 could not be calculated because of growth inhibition was below 50%

Table 9. The values of LC(EC, IC)50 calculated for various type of sewage sludge

values of LC(EC, IC)50 resulting from bioassays provided by Czech legislation are higher or equal to 10 ml L^{-1} at least for one of testing organisms (*Daphnia magna, Sinapis alba,* fresch water algal *Scenedesmus subspicatus and* vertebrate *Danio rerio*) are the waste evaluated as hazardous. Calculated values of LC(EC, IC)50 are in table 9.

In spite of the fact that testing organisms are not the same as define Czech legislation we can predict that in no case sewage exhibit property labelled H14. Obtained values are above 10 ml L^{-1} in all case. The most sensitive organisms are crustacean especially *Thamnocephalus platyurus.* As environmentally friendly appears DWAS and DSAS – activated sludge which is anaerobic stabilized and dewatered and consequently desiccated. It is possible that these processes decrease amount of some water soluble or temperature instable xenobiotics. The ecotoxicity assays confirmed that no sludge constituted a hazardous waste from ecotoxicological point of view. Our results are in according to recent study concerning ecotoxicity assays of different sludge (aerobic, anaerobic, unstabilised and sludge from a waste stabilisation pond) which confirmed that no sludge constituted a hazardous waste (Fuentes et al., 2006). The other question is if the bioassays of water leaches are relevant to predict ecotoxicity of solid matrices (waste, sediments, sewage). The aim of study conducted on various organisms by (Leitgib et al. 2007; Domene et al., 2008) was to assess applicability and reliability of several environmental toxicity tests, comparing the result of the whole soils and their water extracts. Measured endpoints were the bioluminescence inhibition of *Vibrio fischeri* (bacterium), the dehydrogenase activity inhibition of *Azomonas agilis* (bacterium), the reproduction inhibition of *Tetrahymena pyriformis* (protozoon), and *Panagrellus redivivus* (nematode), the mortality of *Folsomia candida* (springtail), the root and shoot elongation inhibition of *Sinapis alba* (plant: white mustard) and the nitrification activity inhibition of an uncontaminated garden soil used as "test organism". In most cases, the contact ecotoxicity tests conducted on solid matrices indicated more harmful effect of these samples than the tests using matrices extracts.

Organisms	Type of test	Endpoinds	Exposition time	Result	Directiva
		Tests of solid waste			
Eisenia fetida - springtail	acute	mortality	14 days	LC50	ISO 11268-1
Avena saitva, Brasicca rapa - plants	acute	germination, growth inhibition	14 days	IC50	ISO 11269-2
		Test of water leach of waste			
Vibrio fischeri - bacterium	acute	inhibition of luminescence	30 min	EC50	ISO 11348
Daphnia magna - crustacean	acute/chronic	inhibition of mortality	48 h	EC50	ISO 6341
Desmodesmus subspicatus, Pseudokirchneriella subcapitata - algae	chronic	growth inhibition	3 days	EC20	ISO 8692

Table 10. Fundamental battery of ecotoxicity test for ecotoxicological evalution of solid matrices

Direct contact environmental toxicity tests are more reliable and enable better prediction of environmental risk of tested matrices. Based on several studies (Rojíčková et al., 1998; Leitgig et al., 2007; Pablos et al., 2009) resulting in similar findings are in Czech recommended another test inclusive obligatory battery of tests mentioned in Czech Legislation. The direct contact environmental toxicity bioassays are able to follows the requirements of environmental toxicology: reliability, sensibility, reproducibility, rapidity and low cost.

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2

Assessment of Micropollutants from Municipal Wastewater- Combination of Exposure and Ecotoxicological Effect Data for Switzerland

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1. Introduction

Micropollutants (MPs) from municipal wastewater are frequently detected in surface waters and occur in ecotoxicologically relevant concentrations. Therefore a broadly accepted method for the assessment of MPs is needed. Here we propose a procedure for the assessment of MPs from municipal wastewater. The method suggested comprises (1) an approach for the identification of potentially polluted sites, (2) a compilation of a substance list with relevant MPs, (3) (eco)toxicologically based quality criteria, (4) a sampling strategy that considers the input-dynamics of chemicals and (5) a scheme to rate water quality with respect to MP contamination. In the proposed concept the assessment focuses upon those substances found repeatedly in municipal wastewaters (continuous inputs).

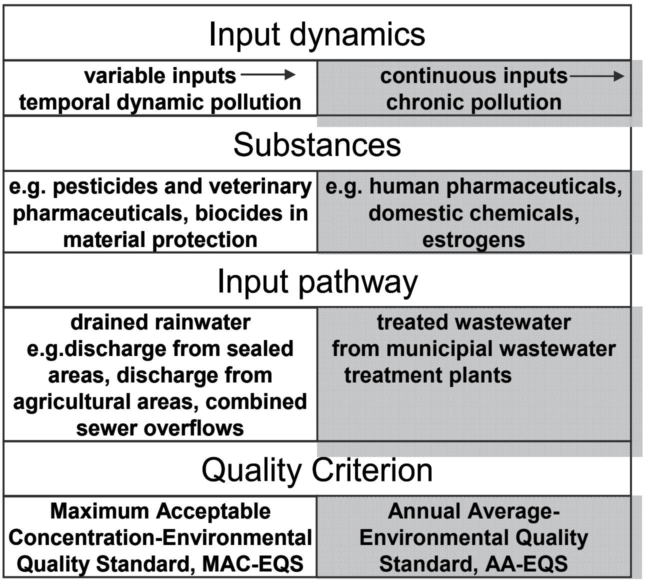
Additionally, we explain how the Environmental Quality Standard (EQS) proposals were derived in accordance with the Water Framework Directive (WFD), and the currently developed Technical Guidance Document for EQS (TGD for EQS). Based on the proposed EQS, we provide a Swiss-wide risk assessment for 6 selected MPs.

1.1 Background

MPs have been found in watercourses at concentrations that can damage the health of animals and plants (Chèvre et al., 2006; Escher et al., 2008; Nadzialik et al., 2010). MPs also pollute important drinking water sources such as lakes, large rivers and groundwater (AWEL, 2007, Loos et al., 2009). Studies have shown that in certain water bodies, including important drinking water sources such as Lake Constance, MPs from municipal wastewater are more numerous and are found at higher concentrations than MPs from agricultural sources (Singer et al., 2009). The assessment and reduction of pollution in surface waters constitutes an ongoing challenge for water protection authorities, especially because no generally applicable procedures are available for assessing water quality with respect to MPs. This project carried out within the Strategy Micropoll Project of the Federal Office for the Environment (FOEN) of Switzerland, developed a possible approach to address these problems. The key points of the proposed approach are presented in this article. The assessment concept covers the following points:

- Identification of relevant substances: compilation of a list of MPs from municipal wastewater treatment plants (WWTP) that are important for Switzerland.
- Derivation of effect-based quality criteria for relevant substances.
- Survey using a sampling strategy that takes into consideration the input dynamics of the relevant substances.
- Procedure for assessing water quality with respect to MPs from municipal wastewater.

The assessment is based on an analysis and description of the sources and input pathways of MPs from municipal wastewater. Therefore it focuses on continuous inputs of MPs and the resulting chronic water pollution (Fig. 1).



Input dynamics	
variable inputs → temporal dynamic pollution	**continuous inputs → chronic pollution**
Substances	
e.g. pesticides and veterinary pharmaceuticals, biocides in material protection	**e.g. human pharmaceuticals, domestic chemicals, estrogens**
Input pathway	
drained rainwater e.g.discharge from sealed areas, discharge from agricultural areas, combined sewer overflows	**treated wastewater from municipial wastewater treatment plants**
Quality Criterion	
Maximum Acceptable Concentration-Environmental Quality Standard, MAC-EQS	**Annual Average-Environmental Quality Standard, AA-EQS**

Fig. 1.Overview of the pollution of surface water with MPs

The proposed approach is based on the chemical and physical surveys of nutrients of FOEN's Modular Stepwise Procedure (MSP) (Liechti, 2010). The following concept for the ecotoxicological assessment of micropollutants from municipal wastewater has been published in January 2011 as a joint report by Eawag and the Swiss Centre for Applied Ecotoxicology (Götz et al. 2011).

1.2 Sources

Thousands of different chemicals with various applications are in everyday use. The main sources for MPs discharged into surface waters via municipal wastewater can be categorized into substances with indoor applications and substances with applications outside of buildings.

Indoor applications:

- *Households* (e.g. dishwashing liquids, detergents, personal care products and pharmaceuticals)
- *Healthcare institutions* (e.g. pharmaceuticals, disinfectants and detergents)

- *Manufacturing and commercial enterprises* (e.g. industrial chemicals, production residues and corrosion protection agents), which are connected to the municipal sewage system. Pollutants from industrial and commercial sources are generally not comparable with those found in household wastewater.

Outdoor applications:

- Green spaces and parks in residential areas (e.g. biocides and pesticides).
- *Flat roofs and buildings envelopes and paints* (e.g. biocides and chemicals used in construction).

Depending on the sewage systems, substances from indoor and outdoor applications may have different input pathways into surface waters.

1.3 Input pathways

The most important input pathways for MPs from municipal wastewater are:

a. with treated sewage from municipal sewage treatment plants
b. through combined sewer overflows during rain (combined systems)
c. through leakage in sewage systems
d. through rain water drains (separation systems)

I. Many MPs found in surface waters originate from the urban drainage system (AFU St. Gallen, 2009; AWEL, 2003; AWEL, 2004; AWEL, 2005; CIPEL, 2008; Giger et al. ; 2006; Hollender et al. , 2007; IKSR, 2006; Ort et al. , 2009; Singer et al. ,2008; Singer et al. , 2009; Singer et al. , 2010), are not or only poorly eliminated by municipal wastewater treatment plants and enter the surface water along with the treated sewage effluent. This is backed by the findings in EU-projects like Poseidon for similar MPs (Alder et al., 2006 in IWA, 2006). For such compounds, the concentrations measured in the water are usually well correlated with the proportion of the treated sewage effluent, especially for frequently and widely-used substances which are used indoors and hence enter the

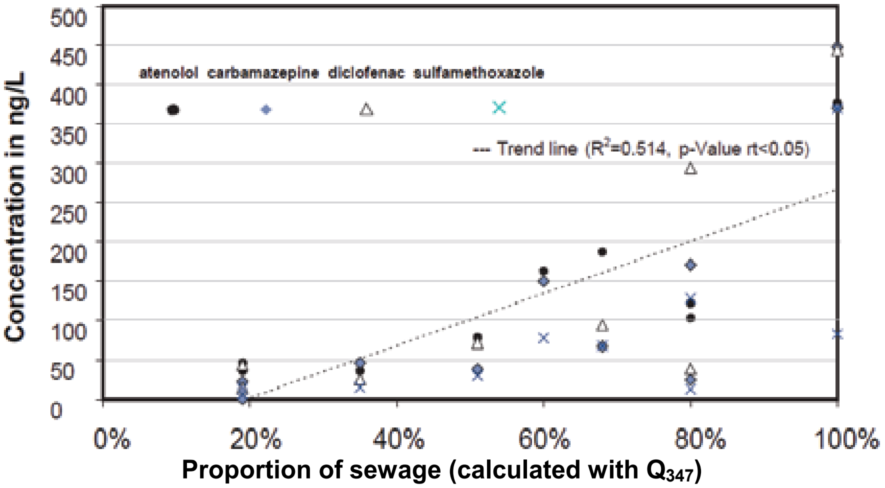


Fig. 2. Calculated concentrations of the drugs atenolol, carbamazepine, diclofenac and sulfamethoxazole with the proportion of sewage at minimum outflow (Q_{347}) (s. section 1.3 b). Measurements are from 2007 and 2009/10.

surface waters mainly through wastewater treatment plants. This is shown in Figure 1 for the drugs atenolol (beta-blocker), carbamazepine (anticonvulsant), diclofenac (painkiller) and sulfamethoxazole (antibiotic). The MPs shown are not eliminated by the wastewater treatment plant, continually enter the surface water and are mainly discharged via treated sewage from the municipal wastewater treatment plants.

II. Today, approximately 75% of urban areas in Switzerland are drained via combined sewer systems (Gujer, 2002). In combined systems, rain water flowing away from residential areas use the same drains as domestic wastewater. Heavy rainfall can cause an overload of sewage systems and treatment plants as their capacity is designed to contain two times the dry weather discharge. When this capacity is exceeded during heavy storms, untreated wastewater enters surface waters directly. In state of the art systems, an annual average of approximately 2.5% of wastewater enters surface waters via the discharge of sewage overflows due to heavy rainfall events. However, this amount can vary greatly, depending on the size and condition of the infrastructure. If a substance is not removed in the wastewater treatment plant and is continuously discharged into the water throughout the year, the proportion of a substance carried by sewage overflow roughly corresponds to the proportion of discharged untreated wastewater. This is, for example, the case with carbamazepine or the artificial sweeteners acesulfame and sucralose, which can therefore be used as tracers for treated wastewater (Fig. 3) (Bürge et al., 2009).

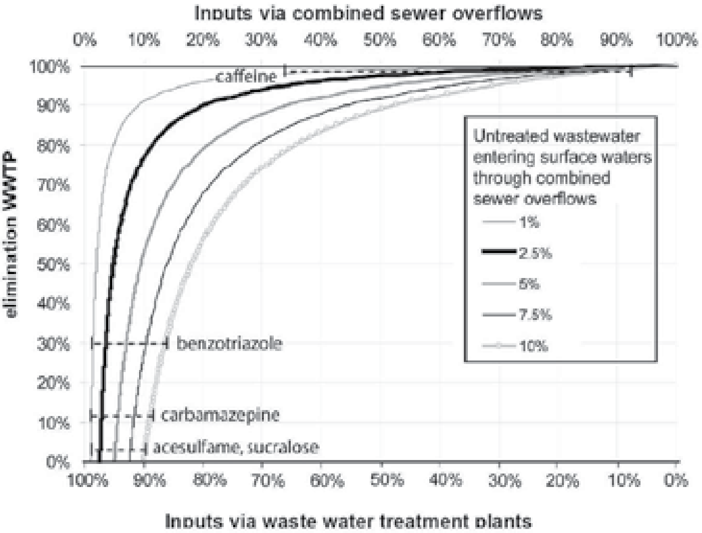


Fig. 3. Calculated proportion of MPs discharged via sewer overflows in combined systems, which is dependent on their elimination in the sewage treatment plant. In the Swiss Lowland, an average of 2.5% of untreated wastewater enters surface waters via the discharge from sewage drains. .

Substances that are predominantly removed by wastewater treatment plants, e.g. caffeine, are discharged into surface waters mainly via the combined sewage overflows and can therefore be used as tracers for the presence of untreated wastewater (Bürge et al., 2006; Wittmer et al., 2010). Inputs via combined sewage overflow also apply to substances with external applications which are mobilized by rain and therefore primarily detected in municipal wastewater even if the combined sewage overflow is active. The Eawag-project REXPO (realistic exposure scenarios) demonstrated that up to 40% of the total amount of mecoprop detected in surface waters, enters through combined sewage overflows (Wittmer et al., 2010). Mecoprop is used as an herbicide in building envelopes and facades and flat roof protection, as well as in plant protection products.

III. Due to leakages in the sewage system, raw wastewater can leach directly into soil, into surface waters and, indirectly into groundwater. It is, however, difficult to quantify losses due to leakages because they depend strongly on the state of local sewer systems (Rieckermann, 2006).

IV. Inputs through rain water drains are not a part of municipal wastewater, but they contain a large number of substances that are similar to the substances found in municipal wastewater from combined systems (substances used outside of buildings). In separated sewer systems, partly polluted rainwater (from roofs, veneers and sealed areas) is transported directly into surface waters via rain water drains. Unlike in combined systems, MPs used outside of buildings flow directly into surface waters with rain water runoff. The advantage of the separating systems is, however, that no MPs from domestic wastewater can be released directly into surface waters, as is the case with the combined systems.

2 Micropollutants

2.1 Range of substances

In Switzerland, thousands of different chemicals are used in different applications and partly enter lakes and rivers. It is impossible to compile a complete list of these substances and their transformation products. In order to evaluate water quality, it is therefore necessary to focus on substances which are relevant to Switzerland’s surface waters.

In order to identify substances relevant for Swiss surface waters, a large data set was evaluated: the results of various measurement campaigns in Swiss surface waters, studies on the behaviour of environmentally relevant substances, publicly available consumer data and various international substance lists (European-Commission, 2006; Freitas et al. , 2004; Hollender et al. , 2007; IKSR, 2006; Keller & Balsiger 2007; Stamm et al., 2008; Stoob et al. , 2005). Based on these data as well as interviews with experts from research, industry, federal agencies and cantonal water protection departments, a list of 250 candidate substances was compiled.. For further prioritisation a procedure described in detail by (Götz et al. ,2010) was applied, which categorises chemicals according to their physico-chemical properties (distribution between water, air and particles), their biodegradability and their emission dynamics.

2.2 Swiss-specific MPs

A list of the main (Swiss-specific) MPs from municipal wastewater was selected from the categorised candidate substance list. The substances identified as Swiss- specific fulfill four criteria:

a. The substance must demonstrably enter surface waters through municipal wastewater.

b. The substance is approved for use in Switzerland by current legislation, i.e. is not prohibited.

c. The substance has properties that indicate that it can be found with average to high probability in the water phase of surface waters.

d. The substance meets at least one of the following three criteria:
   - it has been shown to be widespread in surface waters (>20% of the measurements above the limit of quantification)
   - it has been measured in high concentrations in surface waters (>100 ng/L) and is common in municipal sewage treatment plant discharges (>20%);
   - it has high specific toxicity (e.g. by mutagenicity, carcinogenicity, hormone activity or immunotoxic effect) and as mentioned above meets the condition of relevant entry via municipal wastewater.

The criteria a-c must be fully met, while at least one of the three criteria of condition d) must be met. An example of a substance with a high specific toxicity is the synthetic estrogen ethinylestradiol, which exerts negative effects on the aquatic environment in concentrations below 1 ng/L (Parrot & Blunt, 2005; Wenzel et al. , 1999) .

The 47 Swiss-specific MPs for municipal wastewater selected using the above critera are listed in Table 1a + 1b. The largest group of Swiss-specific MPs from municipal wastewater (22 MPs) are pharmaceuticals. The exposure relevance of pharmaceuticals and other listed

Name of substance	**CAS**	**Group of substance**	*Surface water* # Found / # Measurements	*Surface twater* Average. Concentration. (ng/L)	*Surface water* 90% percentile concentration. (ng/L)	*WWTP effluent* # Found / # Measurements	*WWTP effluent* Average. Concentration. (ng/L)	*WWTP effluent* 90% percentile concentration. (ng/L)
Pharmaceuticals / Drugs								
Atenolol	29122-68-7	Beta-blocker	49 / 75	205	275	18 / 18	843	1160
Azithromycin	83905-01-5	Antibiotic	1 / 43	12	12	18 / 19	175	327
Bezafibrate	41859-67-0	Lipid-lowering drug	10 / 66	24	36	12 / 15	139	251
Carbamazepine	298-46-4	Anticonvulsant	112 / 509	13	43	78 / 78	482	790
Carbamazepin-10,11 – Dihydro-10,11-Dihydroxy	58955-93-4	Transformation product	4 / 4	490	1011	6 / 6	1551	1882
Clarithromycin	81103-11-9	Antibiotic	37 / 74	30	73	32 / 32	276	497
Diatrizoate (= amidotrizoe acids)	117-96-4	Contrast medium	15 / 53	206	482	7 / 10	598	1420
Diclofenac	15307-86-5	Analgesic	77 / 137	65	150	54 / 54	647	1170
Erythromycin 1)	114-07-8	Antibiotic	6 / 28	25	44	17 / 17	42	75
Ethinylestradiol	57-63-6	Synthetic estrogen	4 / 99	5	10	6 / 27	2	3
Ibuprofen	15687-27-1	Analgesic	16 / 137	35	52	54 / 54	394	1439
Iomeprol	78649-41-9	Contrast medium	9 / 53	275	91	9 / 19	380	295
Iopamidol	62883-00-5	Contrast medium	14 / 53	92	51	15 / 19	377	880
Iopromid	73334-07-3	Contrast medium	21 / 53	96	65	13 / 19	876	2460
Mefenamic acids	61-68-7	Analgesic	7 / 28	7	14	10 / 10	870	1658
Metformin	657-24-9	Antidiabetic	13 / 13	713	3057	6 / 6	10347	13427
Metoprolol	37350-58-6	Beta-blocker	24 / 57	20	50	17 / 17	166	322
Naproxen	22204-53-1	Analgesic	22 / 137	37	82	38 / 39	462	678
Sotalol	3930-20-9	Beta-blocker	39 / 74	63	189	21 / 21	435	730
Sulfamethoxazole	723-46-6	Antibiotic	34 / 66	26	59	34 / 34	238	427
N4-Acetylsulfamethoxazol	21312-10-7	Transformation product	5 / 40	3	17	5 / 6	67	157
Trimethoprim	738-70-5	Antibiotic	26 / 74	13	36	42 / 45	100	163

Table 1a. Swiss-specific micropollutants from municipal wastewater: Compilation of analytical data from surface waters and wastewater treatment plant effluents. Data reported in (AFU St. Gallen, 2009; AWEL, 2003; AWEL, 2004; AWEL, 2005; CIPEL, 2008; Giger et al. ; 2006; Hollender et al. , 2007; IKSR, 2006; Ort et al. , 2009; Singer et al. ,2008; Singer et al. , 2009; Singer et al. , 2010) and compiled in the Micropoll-database.

substances is also backed by findings of the EU (Loos et al. , 2009; Alder et al. ,2006 in IWA, 2006) and underlines the need for cross-border risk management. Pharmaceuticals entering surface waters via treated wastewater are generally biologically active substances, with the exception of x-ray contrast media for which only some metabolities and transformation products are discussed to have a biological effect. The second-largest group are 13 MPs with biocidal effect. These substances are used as active ingredients in plant protection products in agriculture or for protection of building materials. They are regularly detected in sewage treatment effluent.

Subsequently, hormone active substances and other substances with environmentally relevant properties are considered. As discussed in chapter 1.3 some MPs without currently-known effects, such as the artificial sweeteners acesulfame and sucralose were also considered as good tracer substances, indicating pollution through municipal wastewater due to their wide prevalence and high persistence in the environment.

Name of substance	**CAS**	**Group of substance**	*Surface water* # Found / # Measurements	*Surface twater* Average. Concentration. (ng/L)	*Surface water* 90% percentile concentration. (ng/L)	*WWTP effluent* # Found / # Measurements	*WWTP effluent* Average. Concentration. (ng/L)	*WWTP effluent* 90% percentile concentration. (ng/L)
Substances with intended biocidal characteristics, which are subject to approval.								
2,4-D	94-75-7	Herbicide	16 / 125	67	53	4 / 6	13	25
Carbendazim	10605-21-7	Fungicide	37 / 73	16	34	17 / 30	81	170
Diazinon	333-41-5	Insecticide	367 / 1211	15	30	40 / 84	173	494
Diethyltoluamide (DEET)	134-62-3	Insecticide	236 / 331	135	120	11 / 55	593	817
Dimethoate	60-51-5	Insecticide	14 / 355	22	34	No data	No data	No data
Diuron	330-54-1	Herbicide	98 / 697	54	70	13 / 34	201	1379
Glyphosate *)	1071-83-6	Herbicide	64 / 162	373	637	No data *)	No data	No data
AMPA	1066-51-9	Transformation product	60 / 162	140	290	No data *)	No data	No data
Irgarol (Cybutryne)	28159-98-0	Herbicide	18 / 878	3	No data	9 / 29	30	58
Isoproturon	34123-59-6	Herbicide	211 / 1001	315	820	11 / 14	12	35
MCPA	94-74-6	Herbicide	56 / 137	40	111	6 / 6	25	44
Mecoprop-p	16484-77-8	Herbicide	100 / 188	45	74	26 / 29	424	765
Triclosan 2)	3380-34-5	Microbiocide	3 / 12	20	31	6 / 6	116	224
Substances with an effect on the hormone balance (hormone active substances, which are not applied as pharmaceuticals/drugs)								
Bisphenol A (BPA) 4)	80-05-7	Additive	44 / 66	840	3440	22 / 25	331	679
Estradiol	50-28-2	Natural estrogens	17 / 92	2	3	18 / 28	3	5
Estrone	53-16-7	Natural estrogens	36 / 116	2	3	26 / 30	15	35
Nonylphenol 3)	104-40-5	Additive	15 / 25	441	1100	7 / 7	267	353
Perfluoroctane sulfonate (PFOS) 4)	1763-23-1	Tenside	No data	No data	No data	No data	No data	No data
Other substances with environmentally relevant properties***)								
Acesulfame	55589-62-3	Food additive	24 / 24	4010	6200	4 / 4	22500	30700
Benzothiazole 4)	95-16-9	Additive	4 / 4	373	862	6 / 6	494	662
Benzotriazole	95-14-7	Corrosion preventative	366 / 382	1230	2990	41 / 41	12881	17300
EDTA **)	60-00-4	Complexing agent	202 / 248	2820	5380	10 / 10	20930	30290

Methylbenzotriazole	136-85-6	Corrosion preventative	303 / 331	249	516	30 / 30	1140	1950
NTA **)	139-13-9	Complexing agent	183 / 253	2890	5800	10 / 10	5370	6930
Sucralose	56038-13-2	Food additive	12 / 13	540	1039	6 / 6	4600	6523

1) Erythromycin is quickly transformed into erythromycin-H_2O. The quantitative analysis is problematic

2) Triclosan adsorbs) relatively strongly (>75% on sludge). The analysis of triclosan is difficult (Singer et al. 2002).

3) For Nonylphenol (NP) only values measured since 2006 have been considered (Ban on certain products with NP from 1.8.2006, ChemrrV)

4) Bisphenol A, PFOS and Benzothiazole are ubiquitous substances. The analysis is difficult due to blank values.

*) Glyphosate is classified with the EPI-Suite (US-EPA, 2007)], as «ready-biodegradable» and would not be considered in accordance with the procedure described above. Measurements show, however, that it occurs in the environment. Glyphosate is one of the best-selling pesticides in the world, frequently applied in residential areas and found in surface waters in the µg/L area (Battaglin et al. , 2005). These high concentrations are not so much explained by the environmental properties of Glyphosate . Due to the very high application a pseudo-persistence explain the high environmental relevance and is also indicated by the transformation product AMPA.

**) EDTA and NTA are classified with the EPI-Suite ,(US-EPA, 2007) as «ready-biodegradable» and would not be considered in accordance with the procedure described above. Measurements of EDTA and NTA in surface waters show, however, that these substances get into surface waters, although the persistent criterion has not been met in accordance with the categorisation according to Götz (Götz et al. ,2010). Therefore the list has been expanded.

***) An environmentally relevant property can also be, for example, a high persistence in addition to toxicity.

No data: No data available

Table 1b. Swiss-specific micropollutants from municipal wastewater: Compilation of analytical data from surface waters and wastewater treatment plant effluents. Data reported in (AFU St. Gallen, 2009; AWEL, 2003; AWEL, 2004; AWEL, 2005; CIPEL, 2008; Giger et al. ; 2006; Hollender et al. , 2007; IKSR, 2006; Ort et al. , 2009; Singer et al. ,2008; Singer et al. , 2009; Singer et al. , 2010) and compiled in the Micropoll-database.

Locally-occurring MPs

Besides the above listed Swiss-specific MPs, additional water pollutants occurring only in certain regions can be of importance. Many substances have strong regional differences in consumption, have specific applications (for example, in industry and manufacturing) or are discharged only locally into a few surface water bodies. It should therefore be clarified during the water quality assessment whether other locally important pollutants are expected to be present in addition to the above Swiss-specific MPs.

3 Protection goals in the Swiss Water Protection Law and ecotoxicological effect assessment

3.1 Protection goals in the Swiss Water Protection Law

The Swiss Federal Water Protection Law (Swiss Federal Water Protection Law (GSchG), 2008) aims to protect waters from harmful effects. Harmful effects can be caused by pollutants which affect the structure or functioning of surface waters.

A good analysis of the protection goals can be found in (Häner et al. ,2010 and Junghans et al. ,2011). The purpose of the Swiss Water Protection Law of 1991 is to protect waters against harmful effects (Art. 1).

- to maintain the health of persons, animals and plants;
- to maintain the natural biotopes of indigenous fauna and flora; and
- to maintain waters suitable to sustain natural fish populations.

An additional important goal is to guarantee the supply and economic use of drinking water. Although this has to be considered when quality standards for surface waters are set, this protection goal will not be discussed any further in this document, whose primary focus is on chemical and ecotoxicological objectives.

Art. 6 of the Swiss Water Protection Law states that it is prohibited to introduce into a waterbody any substances which may pollute such waters, either directly or indirectly.

The Water Protection Law thus provides for comprehensive protection: Waterbodies are to be safeguarded against adverse impacts of all kind to ensure that they can serve a wide variety of functions. The Swiss Water Protection Law applies to all surface and subterranean waters (Art. 2 Swiss Water Protection Law). According to a declaration of the Federal Council (dated 29 April 1987, BBl 1987 II 1104) the protection extends to all natural and artificial public and private waters, including their sources.

Ecological goals for surface waters - and the associated water quality requirements - are specified in the Swiss Water Protection Ordinance:

Annex 1 Swiss Water Protection Ordinance defines ecological objectives for waterbodies. These objectives have to be taken into account for all measures taken under this ordinance (Art 1 Swiss Water Protection Ordinance). For surface waters it is required that pollutants which could enter the water as a result of human activities

- do not accumulate in plants, animals, micro-organisms, suspended matter or sediments
- do not have any harmful effects on the biocoenoses of plants, animals and micro-organisms and on the utilisation of the water
- do not interfere with the biological processes making possible the fulfilment of the basic physiological needs of plant and animal life, such as the metabolic processes, the reproductive processes and the olfactory orientation of animals.

Additionally, the Swiss Water Protection Ordinance also requires that pollutants which could enter the water as a result of human activities occur in a water body :

- at concentrations that are within the range of natural concentrations where they are already present naturally
- at near-zero concentrations where they are not naturally present.

The latter two requirements are based on relevant international agreements (such as the Convention for the Protection of the Aquatic Environment of the North-East Atlantic, OSPAR Convention), including those which aim to prevent and eliminate pollution of the aquatic environment by ceasing or phasing out discharges, emissions and losses of priority hazardous substances, with the ultimate aim of achieving concentrations in the aquatic environment near background values for naturally occurring substances and close to zero for man-made synthetic substances. In general, the protection goals of the Swiss Water Protection Law and the EU Water Framework Directive (EU 2000, WFD, WRRL, RL 2000/60/EG) are quite similar.

3.2 Numerical requirements for water quality and effect-based Environmental Quality Standards (EQS)

For MPs from municipal wastewater, to date, numerical requirements do not exist for most MPs in municipal wastewater, with the exception of pesticides (active substances in plant protection products and biocidal products) , which currently have a non-effect based limit of 0.1 µg/L (Water Protection Ordinance (GSchV, 2008)).

Effect-based numerical requirements for water quality are designated in conjunction with the EU WFD as Environmental Quality Standards (EQS). The aquatic environment can be affected by chemical pollution both in the short and long term, and therefore both acute- and chronic-effects data should be used as the basis for establishing the EQS. In order to ensure that the aquatic environment and human health are adequately protected, EQS expressed as an annual average value, should be established at a level providing protection against long-term exposure, and maximum allowable concentrations should be established to protect against short-term exposure (European Commision 2008).

In order to remain consistent with previous national and international Swiss-relevant publications , AA-EQS are used synonymously with Chronic Quality Criteria (AA-EQS =

CQK) and MAC-EQS are used synonymously with Acute Quality Criteria (MAC-EQS = AQK) respectively. AA-EQS must be derived as protection for the effects of long-term exposure and MAC-EQS against the effects of short-term exposure. To allow an overview about the general EQS situation, the main conclusions of The Society of Environmental Toxicology and Chemistry (SETAC) from recent workshops are listed.

General situation of EQS setting/derivation (this paragraph is based on the workshop conclusions and recommendations of the SETAC concerning EQS derivation (Crane et al. , 2010):

Current practice is for each country to derive their own EQS on the same substances, often using methods that differ only slightly. (Crane et al., 2010). This is highly inefficient and a waste of resources. To improve the situation, it is recommended that the advantages and disadvantages of internationally sharing EQS data and adopoting the same derivation strategies should be examined. This recommendation is based on the following considerations:

e. Even after more than 30 years of work, most countries have developed fewer than 50 EQSs for the aquatic environment.
f. Development of a single EQS usually requires at least 2 to 3 years and can cost US$ 50K to US$ 150K ≈ 112K Euro (exchange rate of January 2011) or more depending on data availability, levels of uncertainty that must be resolved, and any economic or social controversies about the substance.
g. Most countries have similar priority substances.
h. Duplication of work is wasteful; there is great potential for collaboration.
i. Most EQS derivation procedures are similar, so there is potential for international harmonization.
j. Pollution often straddles national borders.
k. Industry and trade are multinational; that is, sources of pollution are international.

Currently in Europe, several multi-national meetings are are scheduled, organized with the intention of harmonizing EQS derivation in different countries and EU member states. The EU Commission coordinates the work of Expert Group on Review (a sub group of Working Group E (WG E) which is tasked with the prioritization of substances and associated EQS derivation for candidate priority substances. The informal Multilateral Group (MG) promotes the exchange of knowledge between the European risk assessors working for the national authorities on Specific Pollutants. This leads to a combination of different national interests and stakeholder interests for the different chemical groups (e.g. plant protection products, biocides, pharmaceuticals, industrial chemicals). Both meetings are very important to reduce unnecessary effort and inefficiency in EQS derivation (see comments of Crane et al., 2010 above).

3.3 Derivation of effect based quality criteria

The EQS-proposals presented here were derived according to the Technical Guidance Document for Deriving Environmental Quality Standards (European Commission, 2009), which is the current technical guidance document of the EU WFD. A short summary of the requirements and validity check of ecotoxicological effect data can be found in Matthiessen et al. (2009). The data used should:

- Be reliable and relevant (e.g., generated according to test guidelines or well documented in open accessable literature to achieve validity and relevance criteria)

- Have been assessed using proper statistical analysis methods
- Avoid unrealistically high test concentrations that may create artifacts
- Be based on experiments in which test concentrations were measured, with measured concentrations used to define endpoints if they differ from nominals by more than ± 20%
- Be fully documented (e.g., conducted to Good Laboratory Practice (GLP))
- Have clear dose–response relationships

The EQS were derived by the Swiss Centre for Applied Ecotoxicology after an extensive review of existing ecotoxicological effect data,and adjustment using data sets of other EU member-states and the use of the current TGD for EQS. The resulting (sub)dossiers are commented on by external experts and checked for plausibility and validity in order to obtain independent multiple reviewed EQS-proposals of Swiss-specific MPs (Fig. 4). Additionally, there is active knowledge exchange with EU risk and hazard assessors to obtain a harmonized and balanced expert judgement for the hazard assessment of the priority substances. Two international working groups are very useful for this purpose: The first, EU Working Group E (WG E) on Chemical Aspects of the Water Framework Directive, is developing EQS-proposals for priority substances with member-states NGOs and stakeholder organizations involvement. The second, an informal Multilateral Group (MG) founded in 2006, in which Member States share their experiences and aim to develop common approaches to setting Specific Pollutant EQS values. In both groups some of the newly identified Swiss relevant MPs (table 1a, 1b) are currently assessed and it seems useful to start with similar or identical proposed EQS to allow a border crossing, harmonized risk management.

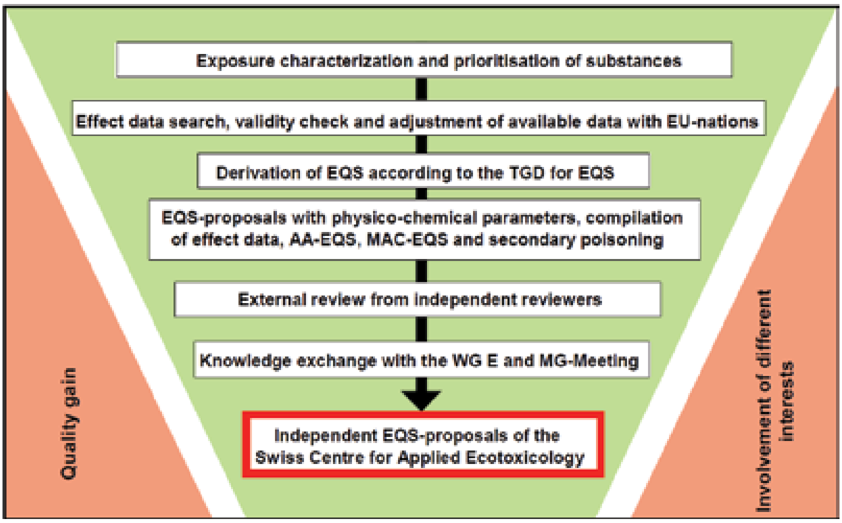


Fig. 4. Steps in the development of an EQS-proposal in Switzerland

For substances for which EQS exists or existed in EU member states, a comparison of the ecotoxicological effect data was made. These effect data were tested for validity (Klimisch et al. , 1997; Matthiessen et al. , 2009) and supplemented by valid up-to-date studies. Proposals for EQS are shown in Table 2. The proposed values are still provisional and will undergo an additional evaluation phase before being finalized. An overview of the currently proposed EQS- can be found at:
http://www.oekotoxzentrum.ch/qualitaetskriterien

As mentioned above, there are some substances for which EQS are being derived for both Switzerland and the EU. The quality criteria of 17-alpha-Ethinylestradiol, 17-beta-Estradiol, Diclofenac, Ibuprofen, PFOS and Cybutryne are currently discussed in the WG E.

Name of substance	**CAS**	**MAC-EQS-proposal**	**AA-EQS-proposal**
Drug / Pharmaceutical			
17-alpha-Ethinylestradiol	57-63-6	no quality criterion proposed	0.037 ng/L
Atenolol	29122-68-7	330 µg/L	150 µg/L
Azithromycin	83905-01-5	0.09 µg/L	0.09 µg/L*
Bezafibrate	41859-67-0	76 µg/L	0.46 µg/L*
Carbamazepine	298-46-4	2550 µg/L	0.5 µg/L
Clarithromycin	81103-11-9	0.11 µg/L	0.06 µg/L*
Diclofenac	15307-86-5, (15307-79-6)	700 µg/L	0.05 µg/L*
Ibuprofen	15687-27-1, (31121-93-4)	23 µg/L	0.3 µg/L*
Mefenamic Acid	61-68-7	40 µg/L	4 µg/L*
Metoprolol	37350-58-6	76 µg/L	64 µg/L
Naproxen	22204-53-1, (26159-34-2)	370 µg/L	1.7 µg/L(*)
Sulfamethoxazole	723-46-6	2.7 µg/L	0.6 µg/L
Trimethoprim	738-70-5	1100 µg/L	60 µg/L
Further substances with environmentally relevant properties			
Benzotriazole	95-14-7, (273-02-9)	120 µg/L	30 µg/L
Methylbenzotriazole	29878-31-7, 29385-43-1, (64665-57-2)	200 µg/L	75 µg/L
EDTA	60-00-4	12100 µg/L	2200 µg/L
NTA	139-13-9, (5064-31-3)	9800 µg/L	190 µg/L
* For these substances a secondary intoxication risk could exist that has not yet been considered numerically. at: http://www.oekotoxzentrum.ch/qualitaetskriterien the updated proposals are available			

Table 2. Proposals for quality criteria of selected Swiss-relevant substances derived according to the TGD for EQS and partly reviewed and verified by external experts.

4. Assessment concept

Figure 4 shows an outline of the various steps of the assessment concept. The individual steps are detailed below.

4.1 Estimation of pollution from municipal wastewater

Assessing concentrations of MPs in surface waters is more time- and cost-intensive than other surface water monitoring, for example those relating to the nutrients (Liechti, 2010). of water pollution. As a basis for assessing water quality using trace analysis methods, an outline should be compiled with regard to expected water pollution by MPs from municipal wastewater. For a first assessment the following methods should be taken into account:

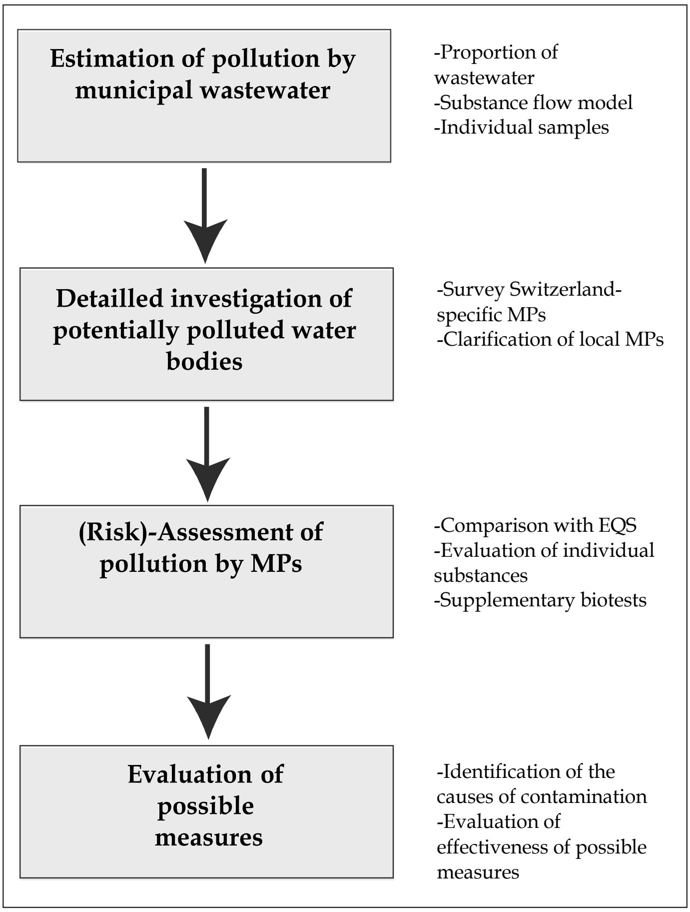


Fig. 5. Outline of the assessment concept

- Identification of the proportion of wastewater in individual surface waters with minimum discharge (Q_{347}): The ratio of wastewater can either (1) be estimated from the population connected to the surface waters via the urban sewer system or (2) be calculated from the measured wastewater discharges from municipal wastewater treatment plants. Detailed information can also be considered, such as discharges from the combined sewage system during rain events, rain water drains in separated sewer systems, and discharges from industry and factories.

- Focusing measurement campaigns using grab samples: Grab samples are collected from specific locations (e.g. below the discharge point of larger wastewater treatment plants) and examined for substances of concern in urban drainage systems, e.g. a selection of the Swiss-specific compounds presented above. When identifying the potential for contamination by MPs in surface waters, specific data relating to the local drainage system should be considered, such as discharges from local point sources.

4.2 Detailed investigation of potentially polluted water bodies

After identification of the potential for contamination by MPs in surface waters, the identified water bodies have to be examined more closely. The list of Swiss-specific MPs can provide guidance for the selection of analytes as AA-EQS have already been derived (Table 2). It should be noted, however, that some substances, such as 17-alpha-ethinylestradiol, are difficult to measure due to the low concentrations at which they occur. Alternative strategies such as measurement of the estrogenicity of samples via integrative biotests or use of passive samplers are currently under development. This substance list can be expanded once more quality criteria have been derived or other substances with environmental relevant properties become known.

Sampling

The sampling strategy proposed here focuses on substances that tend to continuously enter surface waters. Studying MPs with complex input dynamics and various pathways requires a more complex sampling strategy, such as proposed for pesticides by Stamm and colleagues (Stamm et al. , 2006).

In order to measure or monitor the input of organic MPs into surface waters through treated wastewater, the following sampling strategy is recommended:

- Investigation of grab samples from surface waters at least four times a year or more (ideally twelve times a year or more depending on the input characteristics)
- Sampling during the week and not on holidays or weekends (particularly for smaller receiving streams or waste water treatment plants)

Additionally, composite samples from sewage treatment plant effluents can be analyzed. Concentrations in surface waters can then be estimated based on the dilution factor of wastewater in the receiving water body. The advantage of this approach is that most wastewater treatment plants routinely collect 24-hour composite samples of their effluents. This approach minimizes the risk of measurement artifacts associated with grab sampling, however, it ignores the input of MPs from other sources.

Environmental concentration (EC)

In order to assess the water body investigated, a statistical value must be calculated from the analytical data based on a standardised statistical procedure, e.g the environmental concentration (EC). The EC is a critical value. The assessment of the state of the investigated surface water body depends directly on the statistical procedure applied. The EC can be obtained from measured values as follows: arithmetical average value, geometrical average value, median or other percentiles (Liechti, 2010). In order to improve the comparability of the results of the surface water investigations, we suggest considering the 90th percentile for at least twelve samples (Liechti, 2010)

Since the survey of MPs is comparatively expensive, it might not be possible to get twelve measurements. In that case, the average value of the measurement of four seasonal samples normalised at base flow conditions (Q_{347} = the outflow daily average, which is achieved or exceeded on average on 95% of the days, i.e. on average 347 days per year) is proposed to be used as EC. If the difference between the smallest and the largest normalised concentration is higher than an order of magnitude, additional samples or aggregate samples should be taken into consideration.

4.3 Risk assessment of pollution by MPs

A risk assessment is generally made by comparing the environmental concentration (EC) with an EQS. For the evaluation of tested waters according to the above scheme relating to MPs, the EC obtained (90th. percentile, respectively average value) is used.

For continuous inputs of MPs via treated wastewater, the AA-EQS (chapter 3) is particularly relevant as this is how the biotic community can be protected from the consequences of long term exposure:

If the EC is higher than the AA-EQS a non-tolerable risk can be assumed for aquatic communities, as it is usually not known how long these excess concentrations have been in the water. Similar to the MSP, there is a proposed classification into five chemical status categories based on the following levels:

very good /good/moderate/insufficient/poor (see Table 3)

The chemical quality for long-term exposure (AA-EQS) has been achieved for the classifications 'very good' and 'good', and not achieved for the classifications 'moderate', 'insufficient' and 'poor'. The classifications and categories were determined according to Table 3. According to the wide concentration range found in different surface waters, a decadic categorisation scheme was proposed, which covered a large concentration spectrum.

The maximum acceptable concentration (MAC-EQS) aims to protect against possible effects from short-term concentration peaks (European Commission 2009). MAC-EQS can, on the other hand, be used when heavy rain events occur (e.g. storm water events) or when time-varied concentrations of chemicals are expected over a short period (e.g. seasonal applications or chemical accidents). If the short-term EC exceeds a MAC-EQS then a harmful effect on aquatic organisms is possible within the next 48 to 96 hours.

Mixture problems

The evaluation of the MP according to the quality classes (or 'categories') mentioned in Table 3 is based on the ecotoxicity of a single MP alone. Possible combination effects due to the occurrence of several MPs in the same water body are not taken into consideration in the current EQS approach. Since the problem of micropollutant mixtures is not currently recognized in the present single-substance based approach, classifications which include higher-level requirements than the proposed AA-EQS (Factor 10 and 100) are to be taken into account on a precautionary basis and are being proposed by other nations such as the Netherlands (negligible concentration) (van Vlaardingen & Verbruggen, 2007). These allow classification with stricter criteria than the suggested AA-EQS (e.g. AA-EQS/100). The "neglible concentration" (AA-EQS/100) should explicitly protect against potential mixture effects.

Several studies have shown that the consideration of mixture toxicity is an important issue in the context of MP (Kortenkamp et al. 2009 and references therein). It has been demonstrated that the use of NOEC as a toxicologically neglible concentration, which forms the basis for the derivation of the AA-EQS, may not be sufficiently protective when substances occur in complex mixtures. A study by (Junghans et al. 2004) demonstrated that 43 substances, each present at a concentration of as low as a tenth of their respective NOEC, still caused a significant biological effect. Indeed, another study (Baas and Kooijman 2010) demonstrated that, for some water samples, the AA-EQS is not protective when several MPs are present. Currently, several national and international activities are taking place concerning the assessment and regulation of mixture toxicity (e.g. Council of the European Union 2009). Nonetheless, the derivation of sound AA-EQS for single MPs and their degradation products is the basis for every assessment of the water quality and hence an important step to reach a good chemical status in surface waters.

Evaluation		**Criterion/description**		**Compliance with quality criterion**
🟦	very good	The environmental concentration (EC) is 100 times smaller than the quality criterion (AA-EQS)	EC < 0.01 x AA-EQS	AA-EQS passed
🟦		The environmental concentration (EC) is 10 times smaller than the quality criterion (AA-EQS)	0.01 x AA-EQS ≤ EC < 0.1 x AA-EQS	
🟩	good	The environmental concentration (EC) is smaller than the quality criterion (AA-EQS)	0.1 x AA-EQS ≤ EC < 1 x AA-EQS	
🟨	moderate	The environmental concentration (EC) is smaller than the doubled quality criterion (AA-EQS)	1 x AA-EQS ≤ EC < 2 x AA-EQS	AA-EQS exceeded
🟧	insufficient	The environmental concentration (EC) is greater than the tenfold quality criterion (AA-EQS)	2 x AA-EQS ≤ EC < 10 x AA-EQS	
🟥	poor	The environmental concentration (EC) is the same or greater than the tenfold quality criterion (AA-EQS)	EC ≥ 10 x AA-EQS	

Table 3. Evaluation of the chemical water quality for MPs from municipal wastewater (adapted from the Nutrients Module of the FOEN and the modular stepwise procedure) This classification system reflects the chemical quality and not the ecological quality.

An exceedance for one substance could mean that no good ecological status can be achieved in the context of the WFD, because very sensitive species are possibly impacted. A further evaluation of the context between the chemical and ecological status is currently in progress at the European (Environment Directorate-General of the European Commission, DGENV) and national level in the modular stepwise procedure framework (Modulstufenkonzept, MSP). Additional considerations of bioavailability, integrative biotest results and the use of effect directed analysis (EDA), passive sampling strategies and the identification of species

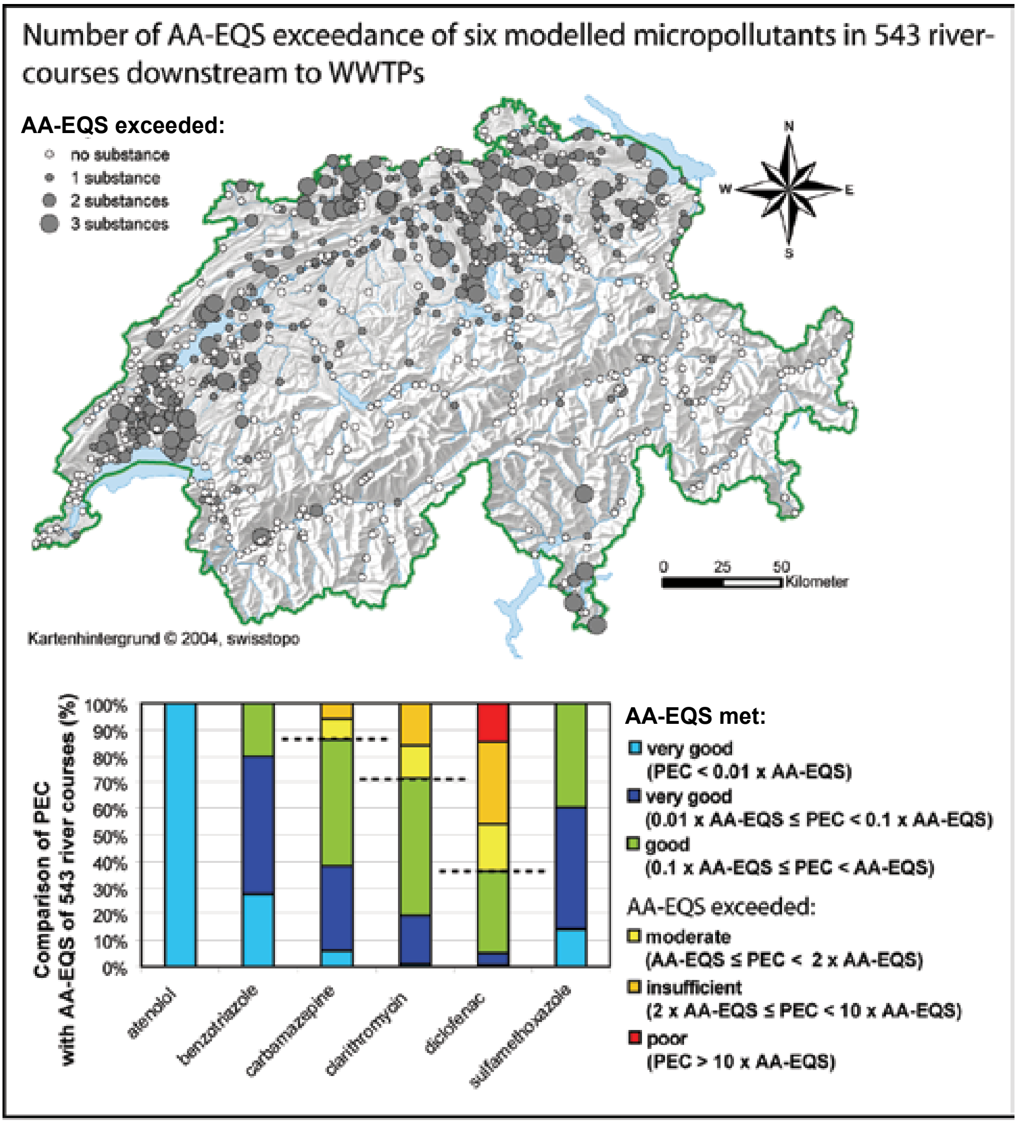


Fig. 5. Evaluation of 543 water sections downstream of sewage treatment plants concerning atenolol, benzotriazole, carbamazepine, clarithromycin, diclofenac und sulfamethoxazole. The environmental concentration (EC) of the MPs examined was calculated by means of the substance exposure model of Ort (Ort et al. , 2007) (PEC = predicted environmental concentration), assuming low flow (Q_{347}) and compared with the chronic quality criteria. The risk assessment of sulfamethoxazole was made with a 5 times lower AA-EQS proposal of 0.12 µg/L than in Table 2 and showed no exceedances.

at risk (SPEAR) seem to be case by case options to provide a more integrative assessment in the future.

During the evaluation of MPs according to the single substance based classifications set out above, it was noticed that the detection and quantification limit could influence the outcome. Should the analytical limit of quantification for a substance be above a category limit, then a classification results as a minimum category. E.g. if a substance is not detectable and if the limit of quantification is between the AA-EQS/10 and the AA-EQS, then the minimum category can be designated "good+" since the actual exposure concentration can be designated as "good" or "very good".

In the Swiss wide Risk Assessment for 6 substances all classes and colours were obtained. Signaling that the chosen classes are appropriate to identify accurately the risks and the safety for single substances.

In 14 % of the investigated cases 3 of 6 substances exceeded the AA-EQS, so there is the possibility for the occurrence of combination effects Further research is needed until other solutions for the mixture problematic are established. For known and monitored substances with a similar mode of action (e.g. specific estrogens, specific herbicides or specific pharmaceuticals like beta-blockers) the addition of risk quotients RQ (RQ= EC / AA-EQS) seem useful to calculate a risk caused by mixture toxicity (Chèvre et al. 2008a, Chèvre et al. 2008b). But in most cases a large number of unknown substances occur and cause mixture effects, therefore the use of mode of action specific biotest monitoring or effect directed analysis (EDA) could lead to an estimation of mixture toxicity. In calculating risk quotients there is always the possibilitiy of errors and deviations on the side of the analytics and the reliability of effect based quality criterion. More risk classes allow a better differentiation in a broader concentration range and, so they could help to avoid an overestimation of a identified risk and enable a better geographical resultion of hot spots or unpolluted areas.

Precautionary target values

Some very exposure relevant and persistent MPs can potentially infiltrate into the ground water used for drinking water. Depending on how much effort is spent on eliminating these substances during drinking water treatment,this is reason for identifying and using a better classification system where chemicals occur above the AA-EQS level. The application of an effect based near-zero concentration and precautionary target values may be appropriate for reducing these concentrations in drinking water relevant water bodies. For surface waters, which are used as drinking water resources, precautionary target-values were proposed by the Waterworks Working Groups IAWD[1], IAWR[2] and the RiWA-Maas[3], who provide drinking water for 106 million people in 17 neighbouring countries (IAWD RIWA-Maas & IAWR, 2008). These values are proposed to be applied to evaluate surface waters used for drinking water, in addition to effect-based AA-EQS. The aim of these precautionary values is to achieve good drinking water quality using only natural preparatory methods for producing drinking water. Persistent substances, which are difficult to eliminate, even if they have no currently known effects, are generally undesirable in drinking water resources and should not exceed 1 µg/L (IAWD RIWA-Maas & IAWR, 2008). For biologically active substances, such as pharmaceuticals, a maximum concentration of 0.1 µg/L is proposed, unless toxicological findings necessitate a lower level (IAWD RIWA-Maas & IAWR, 2008).

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[1] IAWD = Internationale Arbeitsgemeinschaft der Wasserwerke im Donaueinzugsgebiet (International Waterworks Working Group for the Danube catchment area)

[2] IAWR = Internationale Arbeitsgemeinschaft der Wasserwerke im Rheineinzugsgebiet (International Waterworks Working Group for the Rhine catchment area)

[3] RiWA = Vereniging van Rivierwaterbedrijven Maas (Waterworks Association)

4.4 Hormone active effects

There is an urgent need to detect, assess, and reduce effects of hormonally active compounds and endocrine disrupters in aquatic systems, as reflected in national research programs like the Swiss NRP 50 "Endocrine Disruptors" and its consensus platforms (Schweizer Nationalfonds, FNSNF, 2007). As a medium-term measure, the EU strategy on endocrine disruptors (SEC, 2007) uses the Endocrine Disruptor Testing and Assessment (EDTA) Task Force of the Organisation for Economic Co-operation and Development (OECD) along with other research activities. In particular, the test methods of the OECD that are currently being validated or which have already been validated may contribute to a better understanding of the extent of endocrine disruption, especially if they are applied on environmental samples and in the context of risk-assessment strategies, for instance in waste water treatment. Further standardisation of such methods for regulative applications is recommended. (Kase et al., 2009).

In addition to the detection and evaluation of single substances with chemical analytics, the integrative effect detection using *in-vitro*-biotests is recommended for hormone active MPs. In particular, this is desirable for estrogen-receptor binding substances since their quality criteria are analytically difficult to monitor due to the low effect concentrations (< 1 ng/L). With *in vitro* testing the entire estrogen receptor binding potential of an environmental sample can be evaluated with a 17-beta-estradiol equivalent, for example, with the Yeast Estrogen Screen (YES Test) and various reporter gene systems with human cell lines (van der Linden et al. , 2008, Wilson et al. , 2004).

An evaluation of sensitive effect-based, easy-to-manage, economical and easy-to-interpret biotests for estrogenic effects for use by enforcement authorities or by private laboratories is also being sought in the ecotoxicology module of the MSP. A comparative assessment for the applicability of 15 (10 *in vitro* and 5 *in vivo*) biotest procedures for the detection of hormone-active and reproduction toxic effects were carried out on behalf of the Swiss Centre for Applied Ecotoxicology (Kase et al. , 2009). Some biotests are already quite advanced in the validation process of the OECD; others are also in the preparation phase for the ISO-level standardisation necessary for environmental sample testing so that probably within the next three to four years certified, standardised procedures for environmental sample testing can be expected.

5. Swiss-wide situation analyses of selected MPs

Using the mass flow model developed and presented in (Ort et al. , 2007) and recent use data, a Switzerland-wide overview was produced for six MPs, for which AA-EQS were derived: atenolol, benzotriazole, carbamazepine, clarithromycin, diclofenac and sulfamethoxazole. It was thereby assumed that the substances observed enter the surface waters continuously via treated wastewater. For the six selected MPs, good prediction accuracy could be demonstrated (Ort et al. , 2007).

Figure 5 shows the expected Swiss-wide pollution of the water sections downstream of WWTPs at base flow conditions (Q_{347}), based on predicted environmental concentrations (PEC) for six MPs. AA-EQS limits were not exceeded in any of the 543 sections modelled for atenolol, benzotriazole und sulfamethoxazole. The AA-EQS of carbamazepine, clarithromycin und diclofenac were exceeded in different quantities, mainly in the Swiss lowlands. In 14% of the water sections modelled, the EC of the three MPs lie above the AA-

EQS. These water sections could, for instance, be prioritised for more detailed studies. A procedure and further steps in line with the assessment concept detailed above should be checked and evaluated individually.

6. Discussion and outlook

The assessment concept presented here focuses mainly on the input of MPs via treated wastewater and shows possible methods to monitor and evaluate them. Certain aspects, e.g. the selection of relevant substances, can be used for other input pathways than input through wastewater treatment plants, namely the discharge of combined sewer overflows, leakages in the sewer system and, to a certain extent, to inputs through rainwater drains. The procedure presented permits an evaluation of single water sections for single MPs from municipal wastewater similar to the evaluation of other parameters such as nutrients or heavy metals which are regulated in the Water Protection Ordinance (GSchV, 2008). The input dynamics of MPs from municipal wastewater via combined sewer overflows or rain water drains cannot be compiled with the concept proposed. At best it can help determine fundamental contamination by these substances.

In further projects dynamic inputs, such as diffuse inputs of pesticides from agriculture or substances from street drainage systems, should be characterised and investigated.

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3

Water Toxicity Monitoring using Optical Oxygen Sensing and Respirometry

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1. Introduction

Approximately one third of available freshwater is currently used for agricultural, industrial or domestic purposes. This results in contamination of the water with a wide range of pollutants originating from ~300 million tons of compounds used in industrial and consumer products, ~140 million tons of fertilizers, several million tons of pesticides, 0.4 million tons from oil and gasoline spillages (1). To tackle the emerging threat of contamination and depletion of freshwater stocks, large initiatives such as the EU Water Framework Directive (WFD) (2) have been established. The WFD is concerned with "scope of water protection to include all waters, to set clear objectives in order that a "good status" be achieved."

Successful realization of such projects, and of the other environmental monitoring tasks, is linked to the availability of techniques for detailed toxicological assessment, screening and monitoring of large number of chemical and environmental samples, plus validation and wide deployment of such techniques.

Conventional toxicity tests with higher animal models such as rodents or primates based on the determination of lethal doses of toxicants (3) have limited use, due to their ethical constrains, low speed and high costs. Other systems include bioluminescent test for the presence of toxic compounds using freeze dried luminescent bacteria *Vibrio fischeri* (formerly called *Photobacterium phosphoreum* (4)) found in the marine environments (5) and functioning via an endogenous flavin monooxygenase enzyme luciferase. *V. fischeri* provided the basis for several commercial kits such as Microtox® (Azur Environmental, Carlsbad, CA), Mutatox® (with dark mutant of *V.fischeri*) (6), Deltatox® (portable, without temperature control), which have been extensively validated (7, 8) and accepted as a standard method by International Standard Organization (ISO) (9). Although providing good sensitivity, short assay time and simplicity, these tests are limited to just one strain of simple prokaryotic test organism and to samples that do not interfere with luminescent measurements. Samples that are turbid, absorb light or quench luminescent reaction can interfere the assay and cause measurement problems and invalid results.

The need to find alternatives to expensive, space, time and labour consuming toxicity tests using aquatic and terrestrial species has led to the development of alternative methods. Thus, ethical (10) and regulatory issues (11) are favouring the use of animal models such as bacteria (12), small vertebrates (zebrafish *Danio rerio*) (13), invertebrates (the fruit fly *Drosophila melanogaster* (14), and brine shrimp *Artemia salina* (15). Daphnids, particularly *D.*

magna, show widespread occurrence, ecological significance (broad distribution and important link in pelagic food chains), parthenogenetic reproduction, short life cycle and sensitivity to a broad range of chemicals and environmental pollutants. As a result, daphnids are regarded as general representative of freshwater zooplankton species (16). Due to the ease of laboratory culture, discrete growth, small size, high fecundity, low cost and minimal equipment required for bioassays, they have been accepted as standard invertebrates for aquatic toxicologists for testing chemicals (17, 18), surface water and effluents (19) (for example standard EPA toxicity test using *D. magna* (20)). Rapid tests for acute toxicity have been described based on the assessment of immobilization (or mortality) of *D. magna* (17), however they show reduced sensitivity.

Danio rerio (zebrafish) is another widely used test organism which relates to vertebrate animals. Zebrafish embryos are transparent and develop externally. During early phases of development they readily absorb chemicals, thus permitting the *in vivo* assessment of toxic effects of the latter on internal organs and tissues (21). The fish is easy to maintain and breed, its fecundity is high (each female can produce 100 - 200 eggs per mating) providing large numbers of animals for high throughput screening (HTS) applications (21). Small size makes zebrafish one of the few vertebrates that can be analysed in 96- or even 384-well plates, which is essential for HTS of compound libraries (21). Application of potential toxins and drugs to zebrafish is simple: through skin and gills by simply diluting low molecular weight compounds in the surrounding media, or highly hydrophilic compounds can be injected directly into the embryos. Again, most of toxicity tests using zebrafish (and *D.magna)* rely on simple mortality assessment (LD_{50}), thus being subjective, prone to false-positives and providing limited information and specificity. They are not very adequate for predicting toxic effects in humans and higher animals.

Monitoring the rate of oxygen consumption - a sensitive metabolic biomarker of aerobic organisms - has high potential for toxicity testing. Early respirometric studies with daphnids employed Strathkelvin respirometer (22), calibrated oxygen electrode in BOD bottles (23) or in a through-flow system (24), or chemical Winkler method (25) where the amount of dissolved oxygen reflects the biological activity of water masses. However, these techniques are rather labour-intensive and slow, require high numbers of test organisms, and have limited sample throughput. In contrast, optical oxygen respirometry employs a fluorescence/phosphorescence based oxygen sensing probe – a soluble reagent which is added to the sample (26). Probe fluorescence is quenched (reversibly) by dissolved oxygen, and depletion of the latter due to animal respiration causes an increase in probe signal, thus allowing continuous monitoring and real-time quantification of dissolved oxygen. Fluorescent signal of the probe relates to oxygen concentration as (27): $[O_2]= (I_0-I)/I*Ks\text{-}v$, where I_0 and I emission intensities of the oxygen probe in the absence and presence of oxygen concentration $[O_2]$, and Ks-v = Stern-Volmer quenching constant.

Measurement of probe signal in respiring samples on a fluorescence reader allows monitoring of oxygen concentration, e.g. in a standard 96 well plate (WP). From these data, respiration rates can be obtained for each sample, and changes in animal respiration (fold-increase or decrease relative to the untreated organisms) determined, thus reflecting the effect of the toxicant on the metabolism. This approach has been demonstrated with different prokaryotic and eukaryotic cell cultures and model animals including *Artemia salina* (brine shrimp) *Danio rerio*, *C.elegans* (28),(26). Optical micro-respirometry provides simple, high throughput toxicity testing of various compounds and their effects on test organisms.

In this study, we describe the application of optical oxygen micro-respirometry to the assessment of toxicity of chemical and environmental samples, using *V. fischeri (*prokaryote), *D.magna (*invertebrate), and *Danio rerio (*vertebrate) as test organisms. Representative toxicants were heavy metal ions, organic solvents, marine toxins microcystins (MCs) and WWS. The marine toxin microcystin-LR relates to a group of cyclic heptapeptides produced by cyanobacterial species such as *Microcystis aeuruginosa.* MCs are associated with poisoning of animals and humans during cyanobacterial and algal blooms (29). Due to their widespread distribution, high toxicity and threat to public health, MC levels have become an important parameter in water quality control, environmental monitoring and toxicology. A deeper understanding of the toxic action of MC on cells and higher organisms and development of techniques for their detection in environmental samples are important for ecotoxicology. We describe new methods of analysis of environmental samples for MC-LR type of toxicity using optical oxygen micro-respirometry and *Danio rerio* as test organisms. These tests were subsequently validated with a panel of contaminated water samples. The toxicants were examined for their dose-, time- and organism-dependent patterns of response emanating from such respirometric experiments performed in a simple and convenient 96 WP format. This was aimed to achieve a more detailed toxicological assessment and profiling have a deeper insight into the modes of toxicity.

2. Materials and methods

2.1 Materials

Phosphorescent oxygen sensing probe, MitoXpress™ (excitable at 340-400 nm and emitting at 630-690 nm (30)) and sealing oil were obtained from Luxcel Biosciences (Cork, Ireland). Analytical grade $ZnSO_4$ * $7H_2O$, $CdCl_2$, $K_2Cr_2O_7$, sodium lauryl sulfate (SLS), DMSO and MC-LR were from Sigma-Aldrich (Ireland). Solutions of chemicals were prepared using Millipore grade water. The components for nutrient broth medium were supplied from Sigma-Aldrich (Ireland). Standard flat bottom 96 WP and 384 WP were made from clear polystyrene were from Sarstedt (Ireland). The low-volume sealable 96-well plates, type MPU96-U1 were from Luxcel Biosciences (Ireland).

The gram-negative marine luminescent bacterium *V. fischeri* (strain NRRLB-11177, freeze-dried), reconstitution solution (ultrapure water) and diluents (2% NaCl solution to provide osmotic protection for the organism) were obtained from Strategic Biosolutions (USA). *D.magna* stock was collected from continuous culture at the Shannon Aquatic Toxicology Laboratory (Shannon, Co. Clare, Ireland). *Danio rerio* were obtained from Murray Aquatics, UK.

Effluent samples collected from different sites (EPA license classification) were obtained from the Shannon Aquatic Toxicology Lab. Samples of drinking water contaminated with MCs from reservoirs, lakes, fish ponds (more than 300 samples from over 100 localities) were collected during 2007 summer season within the National monitoring program on toxic cyanobacteria, Czech Republic (31).

V. fischeri culture and exposure to toxicants

The lyophilized bacteria were rehydrated in 10 mL and then cultivated in nutrient medium containing: NaCl (23 g), Na_2HPO_4 (15.5 g), nutrient broth 2 (10 g), NaH_2PO_4 (2 g), glycerol per 1 L deionised water (32). 100 mL cultures were grown in 500 mL flask at room temperature (20°C) and shaken at 200 rpm after inoculation with 1 mL of *V.fischeri* culture. Bacteria proliferation was monitored by measuring the increase of optical density in the culture suspension at 600 nm (OD_{600}). When the culture reached OD_{600} ~ 0.5, it was used in toxicity assays. Cells were enumerated by light microscopy using standard Neubauer haemocytometer (Assistant) and light microscope Alphaphot-2 YS2 (Nikon). Stock of bacteria was used in the experiments at different dilutions or stored at +4°C for up to 1 week.

In a toxicity assay, 135 µL of *V.fischeri* in nutrient broth containing 0.1µM of MitoXpress™ probe were pipetted directly into the wells of standard 96 WP, and 15 µL of toxicant stock were added to each well to give the desired final concentration. Each concentration of the toxicant was prepared and analysed in 4 replicates on the 96 WP. For the 24 h incubation, 9 mL of LB inoculated with bacteria were added to 50 mL reagent tubes (Sarstedt) containing 1 mL of test compound at the required concentration, and incubated at 30 °C. After incubation, samples were diluted to a concentration of 10^6 cells/mL, mixed with the oxygen sensitive probe and transferred in 150 µL aliquots to the 96 WP. In the 1 h incubation assay, 135 µL of *V.fischeri* in LB broth (10^6 cell/ml) containing 100 nM of the oxygen probe were pipetted directly in the wells of standard 96 WP, and 15 µL of toxicant stock were added to each well to give the required concentration.

D.magna culture and exposure to toxicants/effluents

D.magna was maintained in continuous culture under semi-static conditions at 20 °C±2 °C in 1 L beakers in de-chlorinated water, using 16h light/18h dark photoperiod and a density of 20 adults per litre. Dilution water (total hardness 250±25 mg/L ($CaCO_3$), pH 7.8±0.2, Ca/Mg molar ratio of about 4:1 and dissolved oxygen concentration of above 7 mg/L (33)) was used as both culture and test medium. It was renewed three times a week and beakers were washed with a mixture of mild bleach and warm water. Stock cultures and experimental animals were fed daily with *Chlorella sp* algae (0.322 mg carbon/day). The algal culture was cultivated continuously using freshwater Algal culture medium (34). 3-weeks old offsprings of *D.magna* were separated from cultures at regular intervals and used for the production of juveniles (≤ 24 h), which were then used in toxicity tests.

For acute toxicity testing, 20 juveniles (≤ 24 h) were randomly selected and placed in 50 mL glass beakers or plastic tubes (Sarstedt) containing 40 mL of de-chlorinated (fresh) water with different concentrations of toxicants/effluents and without (untreated controls). As in the standard test (33), *D.magna* were not fed during the incubation. Following 24h or 48h incubation, individual organisms were transferred by Pasteur pipette into microplate wells containing medium and the toxicant.

Effluent samples were initially analyzed undiluted using 24 h exposure and a procedure similar to the chemicals (see above). Subsequently, highly toxic samples were analyzed at several different dilutions. In parallel with respirometric measurements, standard toxicity tests (33) were also conducted to determine the percentage of *D.magna*, which become immobilized after the exposure to different effluent concentrations. Corresponding EC_{50}-24 h values were calculated and compared with the respirometric values.

Danio rerio culture and exposure to toxicants/effluents

Danio rerio were raised and kept in a 10 L freshwater tank at 28°C, on a 14 h light/10 h dark photoperiod (35). *Danio rerio* were fed daily with live *Artemia nauplii* and Tropical Flake® food. Spawning and fertilization of unexposed parent fish was stimulated by the onset of first light. Marbles were used to cover the bottom of the spawning tank to protect newly laid eggs and facilitate their retrieval for study. Fertilized eggs were collected from the bottom of the tank by siphoning with disposable pipette, transferred into a 6-well plate (Sarstedt) with

5 mL of water and kept at 28 °C (for 48 h). For toxicity assays, hatched *Danio rerio* (48 hpf) (36) were transferred into the wells of 6 WP containing 5mL of water to which toxicants and oxygen probe were added at the required concentrations. Following incubation (1 or 24 h), individual animals were transferred into wells of a low-volume 96-well plate (Luxcel Biosciences) - one animal in 10 µL of assay medium per well. The plate was then sealed and analyzed in the same way as described above for *D.magna*.

Respirometric Measurements

The MitoXpress™ probe was reconstituted in 1 mL of MilliQ water to give 1 µM stock. This probe stock was added to the media used in the corresponding toxicity assay at the following working concentrations: 0.1 µM for the 96WP and 0.5 µM for Luxcel plates. Respirometric measurements with *D. magna* and *Danio rerio* were conducted in low-volume sealable Luxcel plates using sample volume 10 µL, and with cells - in 96WP using sample volume 150 µL. Optical measurements were carried out on a fluorescence reader Genios Pro (Tecan, Switzerland) in time-resolved fluorescence mode, using a 380 nm excitation and a 650 nm emission filters, delay time of 40 µs and gate time 100 µs.

The required number of *D.magna* were transferred with a Pasteur pipette into each assay well containing medium with probe. To initiate the respirometric assay, samples were sealed with adhesive tape in Luxcel plates or with mineral oil in 96 or 384 WP (100 µL or 40 µL per well). The plate was then placed in the fluorescent reader set at 25 °C (for *D. magna*) or at 30°C (for *V. fischeri*) and measured in kinetic mode.

For animal based assays fluorescent readings in each assay well were taken every 2 min over 0.5-2 h. Measured time profiles of probe fluorescence for each sample were used to determine changes in respiration for each samples relative to control (wells with untreated test organisms). For that, the initial slopes of probe fluorescent signal, which reflects oxygen consumption rate, were calculated for each well and normalized for their initial intensity signal. These slopes were compared to those of the untreated organisms (positive controls, 100 % respiration) and to those without organisms (negative controls, 0 % respiration). Relative changes in animal respiration and EC_{50} values for the toxicants were determined using sigmoidal fits with logged data fit function as logistic dose response and error bars weighting in OriginPro 7.5G software. A one-way ANOVA with a Dunnetts comparison was used to determine if the difference in respiration for each treatment group was statistically significant compared with the positive control. Each assay point was usually run in 4 (*V.fischeri*) or 8 (*D.magna, Danio rerio*) repeats, and each experiment was repeated 2-3 times to ensure consistent results. Concentrations which caused significant change in respiration, (Cmin) were identified by T test with confidence limits of >99 %.

For the *V.fischeri* assay, readings were taken every 10 minutes over 12 h. Calibration curve for *V.fischeri* was produced by plotting the time required to reach threshold intensity versus seeding density of *V.fischeri* in range from $10\text{-}10^8$ cell/mL. Threshold intensity was defined as half maximum signal reached by an average respiration-growth profile (37). Calibration was used to determine the reasonable concentration of *V.fischeri* used in toxicity test afterwards.

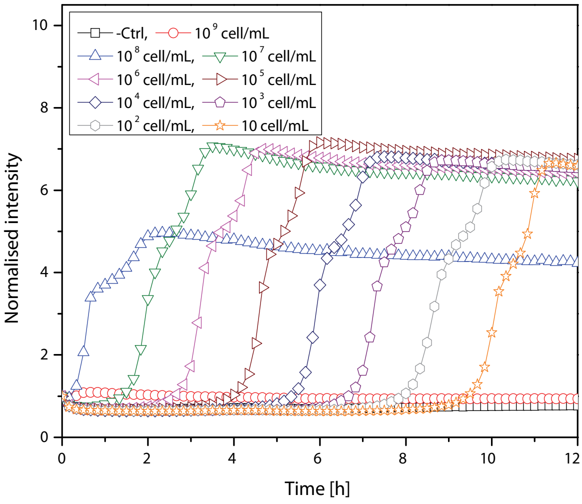
Optical Density (OD_{600}) Analysis of *V.fischeri*

Measurement setup was the same as for the respirometric assay (see above), but no oxygen probe was added to the samples. The microplate was monitored on the Tecan Genious Pro plate reader, measuring absorbance in each well at 620 nm over 8 h periods. Corresponding profiles were then compared with calibration generated with different cell numbers.

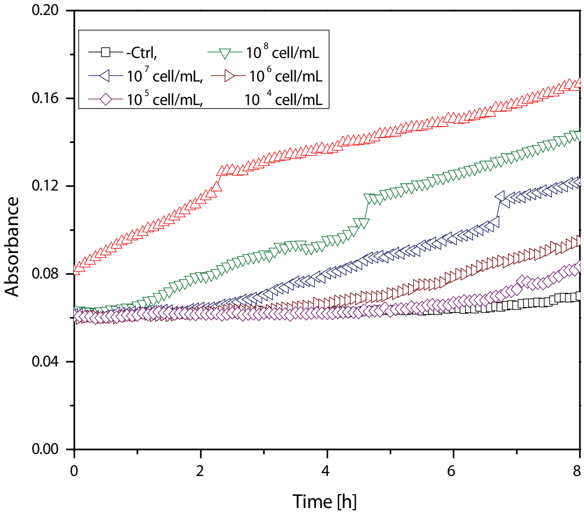
3. Results

Respirometric analysis of model toxicants using *Vibrio fischeri* and *D. magna*

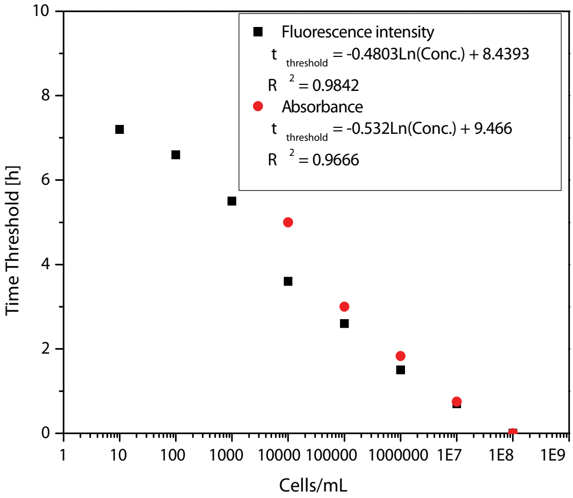
V. fischeri culture was used for toxicity assessment of several types of known toxicants by optical respirometry. For reliable and reproducible measurement of respiration of *V. fischeri* in 96WP, exclusion of ambient air oxygen by sealing the samples with a layer of mineral oil (creates barrier for oxygen diffusion) was used. Respiration profiles of *V.fischeri* seeded at different concentrations in nutrient media containing MitoXpress™ probe and monitored at 20°C are shown in Fig 1a. Profiles of probe fluorescence reflect the process of de-oxygenation of test sample, which is dependent on the initial number of bacteria, their



(a)



(b)



(c)

Fig. 1. Growth profiles of *V.fischeri* seeded at the indicated concentrations in nutrient medium 2 at room temperature (~20°C) and measured on Tecan Genious Pro reader: (a) by oxygen respirometry in time resolved fluorescence mode, (b) by turbidometry in absorbance mode. (c) Calibration curves for quantification of *V.fischeri* by fluorescence intensity (■) and absorbance (●) measurements.

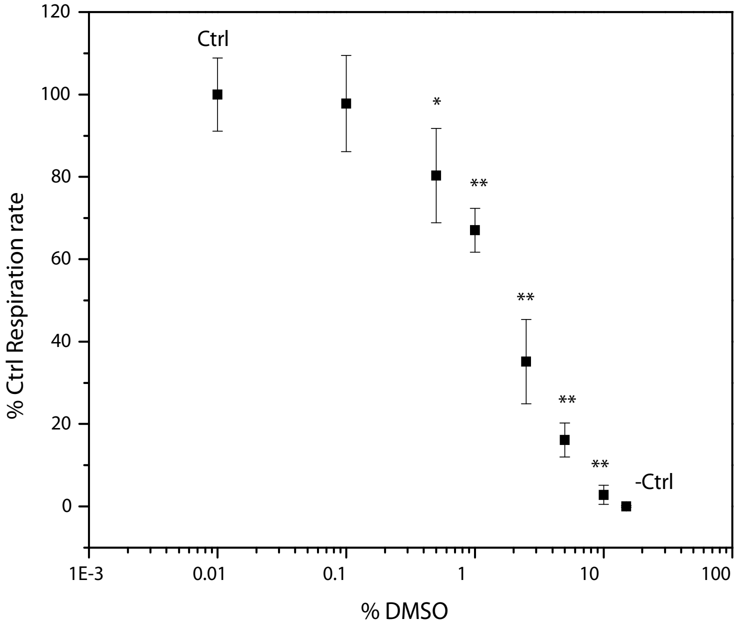


Fig. 2. Processed data (dose response curves) for *V. fischeri* respiration in the presence of DMSO. From such dependence, parameters of toxicity 50 % inhibition values (EC_{50}) were determined, which correspond to the range of toxicant concentrations tested.

proliferation rate and toxicity of the sample. As a result of cellular respiration, dissolved oxygen levels are changing in a sigmoidal fashion from air-saturated at the start of the assay to almost anoxic at long monitoring time. Sample deoxygenation due to bacterial growth is evident as rapid increase of probe signal at high cell concentrations, while low cell concentrations require certain time to induce measurable deoxygenation. Negative samples produce flat signal profiles staying at the baseline level.

Growth profiles of *V. fischeri* were also measured by turbidometric assay (OD_{600}) – the results are shown in Fig 1b. Signal threshold time for *V.fischeri* obtained from fluorescence intensity and absorbance is shown in calibration curve on figure 1c.

For *D. magna*, due to superior performance and greater sensitivity, Luxcel plate with single organism per well were selected for toxicity testing experiments with reference chemicals and effluents. This platform, coupled with a standard fluorescent reader provides low volume and hence more optimal organism to sample ratio giving higher sensitivity of respirometric measurements, and low probe consumption. Other parameters such as temperature (20 ±2 °C) and the age of *D.magna* (≤24h old juveniles) were the same as in the standard method (33). The chemicals chosen for testing were classical reference toxicants. The effect of the toxicants on probe signal (at 0.5 μM) was tested and no interference was observed (data not shown). Following a 24 h exposure, SLS surfactant found in many personal care products (soaps, shampoos etc.) reduced *D.magna* respiration at concentrations of 60 mg/L ($p = 1.1\times10^{-5}$) with EC_{50}-24 h value 33.37 ± 8.72 mg/L (Table 1).

The inorganic toxicant $K_2Cr_2O_7$ is widely used as an oxidizing agent in various laboratory and industrial applications, for cleaning glassware and etching materials commonly used in aquatic toxicity assays (33). After 24 h exposure at 1 mg/L concentration, $K_2Cr_2O_7$ reduced *D.magna* respiration significantly ($p=4x10^{-4}$) compared to positive controls (see Figure 2). Calculated EC_{50}-24 h value was 0.90±0.11 mg/L, which correlates well with literature data, although being slightly lower (Table 1). The respirometric assay also met the criteria of EC_{50}-24 h 0.6 to 2.1 mg/L required for the validation of the conventional test (33).

Toxicant	Standard Assay EC_{50}-24h [mg/L]	Respirometric Assay EC_{50}-24h, ($c_{min.}$) [mg/L]	Standard Assay EC_{50}-48h [mg/L]	Respirometric Assay EC_{50}-48h, ($c_{min.}$) [mg/L]
$K_2Cr_2O_7$	1.12 (33), 3.9 (43)	0.899±0.11, (0.8)	–	–
Sodium lauryl sulfate	50 (43)	64.9±8.28, (60)	–	–
Zn^{2+}	–	4.52±0.58, (4)	1.83±0.07 (44)	1.49±0.14, (0.9)
Cd^{2+}	4.66 (45)	0.63±0.23, (0.3)	1.88 (45), (0.615±0.03) (46)	0.16±0.06, (0.08)

C_{min}: the lowest concentration giving a significant effect (p<0.01).

Table 1. Medium effective concentrations (EC_{50}-24 h/48 h) for different chemicals obtained with *D. magna*.

Exposure to heavy metal ion Zn^{2+} for 24 h had no significant effect on *D.magna* respiration at concentrations 2.2 mg/L (p=0.9) and lower (Fig. 3). However, at 4.4 mg/L and higher it was reduced ($p=7x10^{-4}$) in a dose-dependent manner. 48 h exposure enhanced the toxic effect, which became significant at 0.88 mg/L ($p=1x10^{-3}$) and gave almost complete inhibition at 2 mg/mL. Cd^{2+} ions bind to free sulfhydryl residues, displace zinc co-factors, and generate reactive oxygen species, and exposure to Cd^{2+} results in cellular damage (38). *D.magna* exposed to different Cd^{2+} concentrations after 24 h incubation showed a significant reduction in respiration at 0.3 mg/L ($p=4x10^{-3}$) and 0.6 mg/L ($p<0.001$) (Fig. 3). For 48 h incubation time, significant reduction in respiration was seen at 0.24 mg/L ($p=0.003$). EC_{50}-24 h and EC_{50}-48 h values for Cd^{2+} and Zn^{2+} were determined as 0.63±0.23 mg/L, 0.16±0.06 mg/L and 4.52±0.58 mg/L, 1.49±0.14 mg/L, respectively.

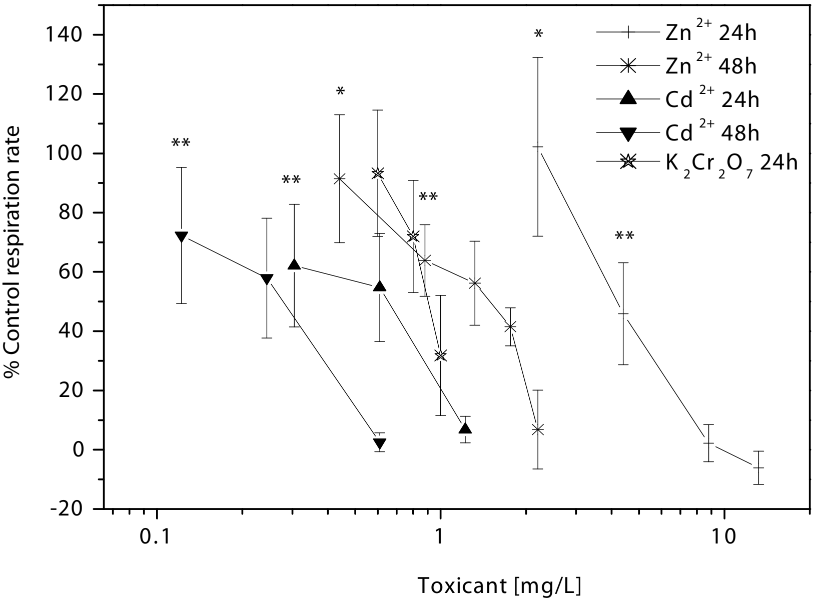


Fig. 3. Dose dependence of toxic effects on *D.magna* respiration of: Zn^{2+} and Cd^{2+} at 24 and 48 h exposure and $K_2Cr_2O_7$ at 24 h, measured in Luxcel plate. T=22°C, N=8.

Analysis of MC-LR toxicity using zebrafish embryos

For animal-based toxicity testing of samples spiked with MC-LR, 48-72 hpf old *Danio rerio* were selected, for which the sensitivity to toxicants and respiration rates appear to be optimal (26). For these fish embryos the culturing procedure is simple and does not require feeding, thus eliminating ethical issues associated with using them in such tests. *Danio rerio* embryos showed very pronounced toxicity to MC-LR at concentrations 0.1-50 nM (Figure 4). Remarkably, after 3h incubation with MC-LR embryos showed a moderate decrease in O_2 consumption, with only those treated with 10 nM MC-LR had their respiration significantly decreased. The toxic effect on respiration was enhanced after 24 hour incubation, with significant drop in oxygen consumption observed at concentrations above 1nM, respectively.

Although *Danio rerio* embryos were not as sensitive to MC-LR as mammalian cells (39), they showed relatively relatively strong susceptibility to MC-LR treatment, with clear time and dose dependent response. This can be explained by the fact that at this stage of development embryos already have a functional liver (40) with cells possessing OATP transporters at their membrane. Freshly isolated fish hepatocytes have shown similar response to MC-LR treatment as rat hepatocytes (41).

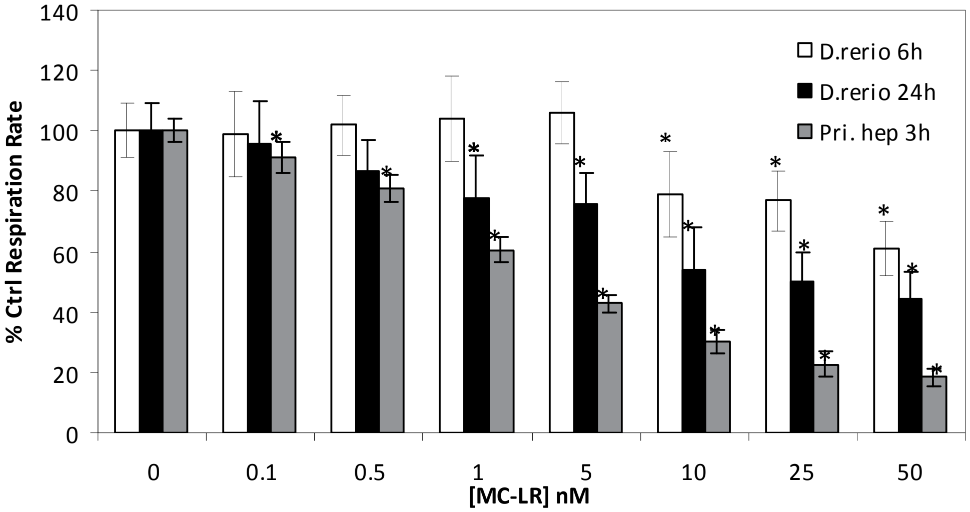


Fig. 4. Respiration of zebrafish embryos and freshly isolated rat hepatocytes (39) in response to MC-LR treatment (relative changes). * - $p<0.05$; ** - $p<0.001$

Analysis of environmental water samples by optical respirometry

To test the efficiency of the respirometric toxicity test with *D.magna* and compare it with standard method, we analysed 10 industrial wastewater samples that were initially examined for their residual toxicity. Initially, samples were analysed undiluted and in a blind manner, i.e. without knowing their source, composition and toxicity in the conventional assay. Thus, a number of toxic samples were identified and subsequently analysed at different dilutions to determine their EC_{50} values. The results were then traced to the origin and possible contamination of each sample and compared with toxicity data produced by the standard test. A summary of effects of all 10 effluents on *D.magna* respiration at different incubation times is shown in see Table 2. The analysis of the samples by the standard test showed toxicity in samples 2-9, which were mainly effluents with elements of metals, pesticides, and pharmaceuticals. Samples 2 and 6 gave EC_{50} values similar to standard test: 6.5% (2) and 14.3% (6), 14.03±4.97% (2) and 14.54±0.74% (6), respectively. Samples 3 and 4 showed a higher sensitivity in standard assay than in respirometric assay, with EC_{50} values of 27.7% (3) and 7.5% (4), and 85.6±37.39% (3) and 19.85±3.82% (4), respectively. Conversely, for samples 5 and 7 the respirometric assay demonstrated higher sensitivity than the standard assay with EC_{50} values 4.01±0.47% (5) and 14.19±6.05% (7), and 7.4% (5) and 41.4% (7), respectively. The inter-assay variation for three independent experiments was in the region of 15-30%. Such variability is quite common for most of the biological assays. It can be compensated for by running appropriate numbers of replicates for each concentration point (N=8 for our systems). Overall, these results show that the respirometric toxicity assay with *D.magna* provides comparable sensitivity and performance with wastewater samples.

The respirometric toxicity assay with *Danio rerio* was also applied to the analysis of water samples contaminated with MCs. 44 hpf *Danio rerio* were incubated for 24h in undiluted field water samples, and then analysed as described above. Two samples were used as a positive (Millipore water), and negative (Millipore water spiked with 100 nM MC-LR) controls, to which the respiration of 17 other unknown samples was compared. The results

Effluent No.	**Standard assay *D.magna***	**Respirometric *D.magna* EC$_{50}$-24h [% vol/vol]**	**EPA Class**	**Industrial Activity Description**
1	>100	ER	5	The use of a chemical or biological process for the production of basic pharmaceutical products
2	6.5	14.03± 4.97	8	The manufacture of paper pulp, paper or board
3	27.7	85.60±37.39	3	The production, recovery, processing or use of ferrous metals in foundries having melting installations
4	7.5	19.85± 3.82	12	The surface treatment of metals and plastic materials using an electrolytic or chemical process.
5	7.4	4.01±0.47	5	The manufacture by way of chemical reaction processes of organic or organo-metallic chemical products
6	14.3	14.54±0.74	5	The manufacture of pesticides, pharmaceuticals or veterinary products and their intermediates.
7	41.1	14.19± 6.05	5	The use of a chemical or biological process for the production of basic pharmaceutical products.
8	20	ER	7	Commercial brewing, distilling, and malting installations.
9	76.9	ND	12	The manufacture or use of coating materials in processes.
10	>100	ND	5	The use of a chemical or biological process for the production of basic pharmaceutical products

ER: Enhanced respiration
ND: Not detectable

Table 2. Toxic effects of industrial effluents on *D.magna*.

are show in Figure 5. We found that the results obtained with fish embryos were similar to those with primary rat hepatocytes (39), although sensitivity of the latter was several times higher. Thus, samples 1, 2, 9, 10, 11, and 12 showed high toxicity. For sample 5 no result was obtained as the embryos were all dead after 24 h incubation. Samples 8, 13, 14, 15, 16 showed moderate to low toxicity, and samples 6, 7, 17 showed no toxicity.

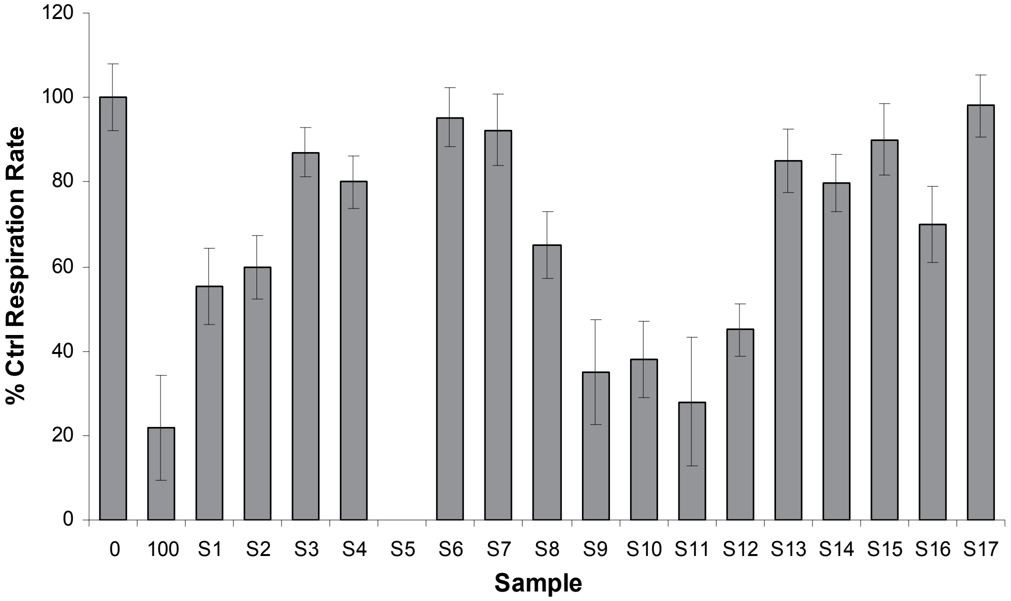


Fig. 5. Changes in respiration of zebrafish embryos treated for 24h with environmental water samples contaminated with MCs (S1-S17). The first two columns correspond to the respiration of embryos incubated with 0 nM and 100 nM MC-LR respectively.

4. Discussion

The results show that fluorescence based oxygen micro-respirometry provides a useful tool for toxicological assessment and screening of water samples. The generic nature of oxygen consumption allows its use as an indicator of viability, metabolic status for various model organisms including *V.fischeri*, *D.magna*, and *Danio rerio*, and sub-lethal toxic effects.

Due to its short life cycle and robust culturing conditions, *D.magna* is a good model organism for rapid preliminary toxicity studies, and such assay can be easily set up even in a small lab. For the analysis of *D.magna* low-volume sealable Luxcel plates specially developed for respirometry are used which can work with one animal per well. Respiration profiles were reproducible and unambiguous. The variation of measured parameters, i.e. respiration rates and EC_{50}-24 h values (see Table 1) is largely attributed to the variation in respiration between individual animals (in line with the variation observed for the other individually tested multicellular metazoans (26)). The results of respirometric tests are comparable with conventional acute toxicity tests.

Danio rerio which has similar organs found in mammals, it is another useful model for toxicity assessment, well established in environmental studies. It is also relatively easy to breed, maintain and produce in high numbers for screening assays. Their size also allows the respirometric assays in Luxcel plates with individual embryos. The assay was used to assess their sensitivity to microcystin-LR, for which susceptibility of *Danio rerio* embryos appeared to be relatively high. However, this assay showed a relatively large variation compared e.g. cell based assays (42), due to significant variation in animal size and embryonic development of organs (MC-LR may influence liver cells differently at different development stages, and liver can metabolise the toxin differently). To generate statistically reliable data, we therefore used higher number of replicates (8-12). Loading *Danio rerio* in Luxcel plates was also a bit cumbersome. Nevertheless, this assay has the potential for the analysis of water samples suspected for contamination with MCs.

Overall, this methodology shows similar sensitivity to standard tests (e.g. Microtox®), and a number of advantages - sample throughput, automation simultaneous measurements, miniaturisation and general simplicity. High flexibility of this platform allows the user to choose test organisms and customize the assay with respect to availability of culturing facilities, the type of samples and toxicants analysed, instrumentation and personnel skills. The possibility to screen large number of chemical and environmental samples highlights the power of this approach. Even with manual liquid handling, one operator can easily generate 100-200 data points per day. The assay is robust and works reliably with complex samples such as effluents or environmental.

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4

Flow-Through Chronopotentiometry in Waste Water Analysis

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1. Introduction

Waste water analysis is still a challenging task for analytical chemistry due to extremely different and complex composition of most of the samples as well due to difficulties in fulfilling the demanded low concentration limits for the analyte species.

For the determination of inorganic species such as metal and semi-metal ions, non-metals and simple compounds titrimetric and spectrophotometric methods are commonly in use. The employment of electrochemical methods is usually limited to potentiometric determinations either as direct potentiometry or potentiometric titration. Voltammetric methods are rarely used though exhibit superior sensitivity, in many instances a satisfactory selectivity and their simple and low cost instrumentation is undisputable. Yet, tedious sample preparation and working procedures, frequent electrode fouling and sample matrix influences have significantly limited the use of voltammetric methods in waste water analysis especially in routine laboratories.

Chronopotentiometric methods, especially in galvanostatic mode seem to be a reasonable alternative to voltammetric ones. By making use of the fast sampling of potential values combined with the memory mapping technique (Hu et al., 1983; Thomsen et al., 1994) their sensitivity equals or even overruns that of voltammetric pulse techniques. The intrinsic disadvantage of chronopotentiometry is the nonlinear (quadratic) concentration dependence of the actual analytical signal – chronopotentiometric transition time, τ – in accordance with the Sand's equation (Bard & Faulkner, 2001). This is of course valid only for conditions the equation was derived for: homogeneous bulk concentration of the analyte at the beginning of the experiment, planar electrode facilitating linear diffusion only, infinite solution thickness, and no movement of the solution relative to the electrode.

If the solution thickness is limited to the value of the diffusion layer and the current density nears to zero, the analyte concentration in the solution decreases virtually equally in the whole bulk and there is virtually no difference between the analyte concentration at the electrode surface and that in the bulk. Hence, the electrode potential corresponds also to the composition of the bulk. In such a case the τ-value is directly proportional to the initial bulk concentration. Thin-layer cells with planar electrodes and solution thickness of 10 – 100 µm can do this. A technically simpler but equally effective way is the use of porous electrodes with pore sizes of the same value.

Linearity at chronopotentiometry can also be achieved by making use of the deposition-stripping approach well known in stripping voltammetry. The analyte species are deposited first at the electrode surface and then stripped by constant current whereas the potential of the working electrode is monitored. The chronopotentiometric transition time is a linear function of the amount of the deposited species, in accordance with the Faraday's laws of electrolysis. In such a way extremely low concentrations can be addressed at least for species which can be reversely deposited at the electrode surface.

By introducing this measurement principle into flow systems, simple but versatile and full automatic analytical systems can be constructed. The heart of the system is the flow-through cell which, especially for routine use and process applications, should comply with strict requirements: long-life, maintenance-free working electrode, robust construction and simple maintenance. Porous electrodes in flow systems offer some unique features making them suitable also for routine applications (Blaedel & Wang, 1980; [a] Beinrohr et al., 1992). Owing to the porous character and large electrode surface, high electrochemical recoveries, up to 100 % can be achieved. The flow system adds an additional advantage, namely the easy exchange of the electrolyte after electrodeposition enabling to strip the deposit to an ideal electrolyte, which minimises the adverse influence of the sample matrix.

1.1 In-Electrode coulometric titrations

Porous electrodes facilitate a special kind of electrochemical measurements, namely thin-layer electrochemistry and coulometry (Bard & Faulkner, 2001). In this type of measurements the average thickness of the solution is beneath the diffusion layer thickness, usually below 100 µm. This can be experimentally achieved by forming a thin solution layer at a planar electrode or by making use of porous electrodes with an average pore size corresponding to the diffusion layer thickness. The former arrangement demands a well-defined geometry of the cell, especially a thoroughly planar electrode. The maintenance of this type of cells and the cleaning and activation of the working electrode are cumbersome. Porous electrodes, on the other hand, can be handled much easier and there are fewer problems with electrode fouling and activation, for this material is cheap and can simply be exchanged if fouled. The electrochemical performance of the porous electrodes, as regards thin-layer properties are virtually the same as those of thin-layer cells with planar electrodes (Bard & Faulkner, 2001).

Thin-layer cells with porous electrodes exhibit some special features:

i. Anodic and cathodic peaks in the cyclic voltammograms of reversible systems appear virtually at the same potentials, especially at lower scan rates. At higher scan rates some shift is observed corresponding to an IR-drop within the cell.
ii. Since the solution forms a thin layer, the electrochemical processes are no more diffusion controlled but the rate is governed by the electron transfer rate, chemical reaction rate and adsorption/desorption phenomena.
iii. The electrochemical changes, especially for reversible systems are extremely rapid and a complete electrochemical change of the whole solution volume can be achieved within a short period of time.
iv. The products of the electrode reaction and consecutive chemical reactions remain within the thin-layer inside the cell and can undergo subsequent electrochemical and chemical changes, such as back-reduction or oxidation.
v. The electrode potential during the electrochemical changes reflects the changes in the chemical composition of the solution and vice versa.
vi. The solution flowing through the porous electrode is intensively mixed facilitating high mass transfer rates which ensure high electrochemical yields.

The above thin-layer properties of cells with porous working electrodes enable to provide some peculiar measurements not available with cells with normal geometry, namely the so called in-electrode coulometric titrations (IECT). The porous electrode alone can be used as a coulometric titration vessel: The virtual sample volume is given by the electrode void volume and the porous electrode serves as the generation as well as the indicator electrode. Hence, there is no need for a separate indication system as in the classical coulometric titrations. The change of the potential of the porous electrode during the coulometric titration with constant current reflects the change of the solution and gives information about the end of the titration.

All in-electrode coulometric titrations are in fact oxidation-reduction titrations and can be applied for species undergoing electrochemical changes at the electrode material and solutions used in the time-scale of the measurement. IECT can be used for species which can directly be oxidized or reduced in the porous electrode. In some instances, the analyte species can be determined by a chemical reaction with electrochemically generated species as known in classical coulometric titrations. The only condition is the appropriate shift of the potential of the porous electrode in the presence or absence of the analyte species in the sample. Hence, the method can hardly be used for species reacting too slowly with the electrogenerated reagent. The material of the porous electrode plays a decisive role in IECT. The electrochemical change of the solvent and the electrolyte should proceed at high overpotentials to ensure low background signals.

The analyte concentration in the sample can be extracted from the electrical charge consumed for the corresponding electrochemical change by making use of the Faraday's laws of electrolysis (Beinrohr, 2001):

$$c = Q/(z\,F\,V) \tag{1}$$

c is the analyte concentration, mol/L
Q is the electrical charge, C
z is the effective charge number
F is the Faraday constant, 96 485.4 C/mol
V is the effective void volume of the porous electrode, L

The consumed charge is given by the product of the applied constant current and the chronopotentiometric transition time, resulting from the potential – time dependence recorded in the course of the titration:

$$Q = I\tau \tag{2}$$

I is the applied current, A
τ is the chronopotentiometric transition time, s.

Obviously, the applied current is not consumed for the electrochemical conversion of the analyte species completely but also for the so called background processes, notably for double layer charging, electrode surface and sample matrix reactions. The measurement of a proper blank may compensate for this influence significantly.

The effective void volume of the electrode can approximately be calculated from the geometry and porosity of the electrode. Its exact determination demands analyses of standard solutions at the same experimental parameters, especially at the same currents as those for the samples. Once calibrated, the electrode can be used until it fouls.

A problem in IECT with porous electrodes arises from the fact that the solution inside the pores of the electrode is connected with the solution bulk and some diffusion could occur during the electrolysis. Hence, the time scale of the measurement influences the effective volume of the sample, the slower the electrochemical process the larger is the effective volume treated.

In principle, the following types of determinations can be done by IECT listed with some possible examples:

i. Acid-base titrations
   Titration of acids: $2\ H_3O^+ + 2\ e^- = H_2 + 2\ H_2O$
   Titration of bases: $4\ OH^- - 4\ e^- = O_2 + 2\ H_2O$
ii. Precipitation titrations
   Argentometry: $X^- + Ag^0 - e^- = AgX_{(s)}$ (X = Cl, Br, I)
   Mercurimetry: $2\ X^- + 2\ Hg^0 - 2\ e^- = Hg_2X_{2\ (s)}$ (X = Cl, Br, I)
iii. Oxidation-reduction titrations
   Determination of iron: $Fe^{3+} + e^- = Fe^{2+}$
   Determination of chromium: $Cr^{3+} + 8\ OH^- - 3\ e^- = CrO_4^{2-} + 4\ H_2O$
iv. Complexation titrations
   Complexation capacity measurements: $Cu^0 + Y - 2\ e^- = [CuY]$ (Cu: copper from the electrode material, Y: complexing species in the sample)

1.2 Stripping chronopotentiometry

For lower analyte concentrations the analyte species can be pre-concentrated at the electrode surface and then "titrated" with constant current: the method is known as stripping chronopotentiometry or constant current potentiometric stripping analysis - PSA (Jagner & Graneli, 1976). All species which can be deposited at the electrode surface and consecutively stripped are available to this method. Electrochemically active metal ions, such as heavy metals and some anions forming negligibly soluble precipitates with the Ag or Hg electrodes can be collected and determined.

Stripping chronopotentiometry with porous electrodes can be employed for the following groups of species:

- Metal ions deposited as elements and then stripped as soluble ions (Zn, Cd, Pb, Hg, Ni, etc.)
- Metal ions depositing as negligibly soluble metal oxides (Mn, Pb, Tl)
- Anions depositing at Ag and Hg electrodes as low-soluble precipitates such as chlorides, sulfides, etc.

The utilization of adsorption phenomena as known from adsorptive stripping voltammetry, has not yet been demonstrated for porous electrodes. This is mainly due to the fact, that adsorbed species tend to remain on the large surface of the porous electrodes and there are problems with the electrode cleaning and regeneration.

An additional feature of the porous electrode is the potential drop inside the electrode bulk arising from the ohmic resistance of the solution and the current flowing through the cell. The higher is the current the larger is the potential drop. In the case of extremely large potential drops some regions of the porous electrode remain virtually inactive and the effective sample volume decreases. Nevertheless, this ohmic drop can successfully be used for the removal of some interference caused by intermetal formation during deposition such as during the determination of Zn in the presence of copper ions (Lazar et al., 1981). Here, the ohmic drop along the electrode bulk may facilitate some sort of electrolytic separation (Nakata et al., 1983) of Zn from Cu and prohibit the formation of the Zn-Cu intermetallic species.

There are several applications of flow-through chronopotentiometry in waste water analysis both in direct chronopotentiometric and stripping chronopotentiometric modes. Some examples for both measurement modes are listed below.

2. Experimental

2.1 Instrumentation

Flow-through chronopotentiometric measurements were carried out by an electrochemical analyser EcaFlow model 150 (Istran, Ltd., Bratislava, Slovakia, www.istran.sk) equipped with two solenoid inert valves, a peristaltic pump, 1 mm inner diameter PTFE tubing and a microprocessor controlled potentiostat/galvanostat. The system is controlled by a PC by making use of a software developed and delivered by the producer. The block diagram of the system is depicted in Fig. 1. The signals were recorded and evaluated by the memory-mapping technique (Hu et al., 1983; Thomsen et al., 1994). The measurement consists of two main steps: i) the background signal is measured first by means of a blank sample, ii) followed by the sample or standard solution giving the signal of the sample or standard: In the stripping mode the sample or standard is pre-concentrated and the cell is rinsed with the carrier electrolyte into which the deposit is stripped. The cell is then rinsed again to remove the stripped analyte species enabling the next run. In the IECT mode the cell is just filled with the sample or standard solution, the flow is stopped and the solution inside the pores of the electrode is titrated by constant current. The background signal is then subtracted from the signal of the standard or sample yielding the corresponding background corrected net signal.

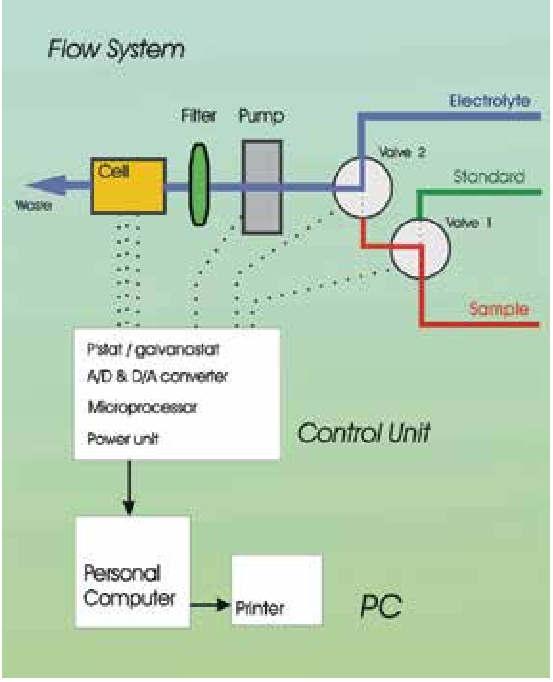

Fig. 1. The block diagram of the flow-through chronopotentiometric analyser

For the sulphide measurement a compact flow-through electrochemical cell of type 104 with Pt auxiliary and Ag/AgCl reference electrodes was used (Istran, Ltd., Bratislava, Slovakia, Fig. 2). The working electrode was a reticulated vitreous carbon plug of 100 ppi (pores per inch) porosity (Electrosynthesis Co. Inc., Lancaster, New York, USA) of 10 mm and 4 mm in diameter and length, respectively.
For Pb and Cr(VI) measurement the cell of type 353c with Pt auxiliary, Ag/AgCl reference and E-53C (for Pb) or E-56C (for Cr(VI)) compact porous carbon working electrodes were used (Istran, Ltd., Bratislava, Slovakia). These electrodes were made of crushed reticulated vitreous carbon material with an effective inner volume of about 20 and 40 µL, respectively.

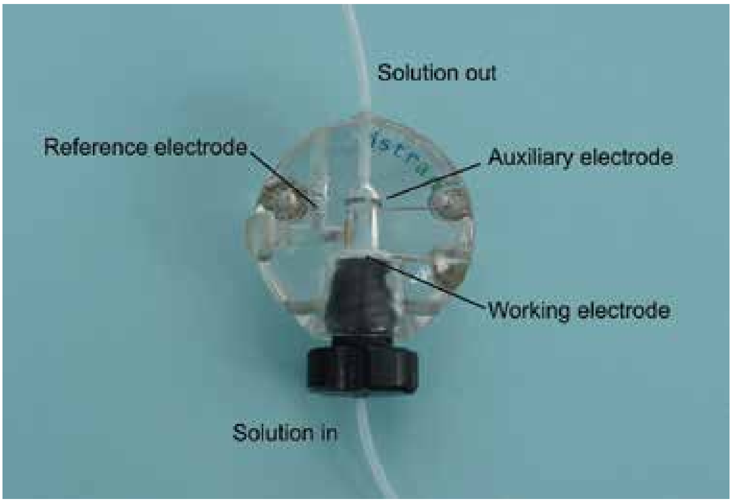

Fig. 2. The 3-electrode flow-through cell

The operation parameters are listed in Tab. 1. All potentials are expressed versus the silver/silver chloride reference electrode built in the cell.

Parameter	*Dimension*	*Sulphide*	*Lead*	*Chromium(VI)*
Deposition potential	mV	-300	-1400	700
Quiescence potential I	mV	-600	-900	700
Quiescence time I	s	10	10	5
Quiescence potential II	mV	0	-900	700
Quiescence time II	s	30	10	0
Terminal potential	mV	-700	-200	200
Regeneration potential	mV	-1000	0	700
Standby potential	mV	-600	-100	700
Stripping current	µA	-500	100	-50
Sample volume	mL	1	0.5 - 5	4
Blank volume	mL	1	0.5 - 5	4
Rinsing volume	mL	4	3	0
Flow rate	mL/min	6	3	6

Table 1. Operation parameters of the flow-through electrochemical analyser for sulphide, lead and chromium determination

The accuracy of the results of Pb and Cr(VI) measurements was checked by GF AAS on the atomic absorption spectrometer Perkin Elmer 5000 equipped with Zeeman background correction. The experimental parameters used were those recommended by the manufacturer.

Analytical-reagent grade chemicals were used in all experiments. Deionised and degassed water was used for the preparation of all solutions.

2.2 Reagents and procedures for sulphide measurement

Carrier electrolyte: 0.1 mol/L Na_2SO_4, 0.01 mol/L CH_3COONa, 0.01 mol/L CH_3COOH.

Electrolyte for sample preparation: 0.1 mol/L NaOH

Hg coating solution: 1.0 mg/L Hg(II) in 0.1 mol/L KSCN.

The bulk standard solution of about 10 g/L S^{2-} was prepared from sodium sulphide ($Na_2S.x H_2O$, containing approx. 60 % Na_2S) in water. Its concentration was determined by iodometric titration. The solution was stable one week if stored in a refrigerator. The calibration solutions were prepared fresh before the measurement by diluting the bulk standard solution in 0.1 mol/L NaOH. The concentration of the standards was in the range of 0.1 to 0.5 mg/L. The calibration solutions should be used immediately after preparation.

The Nafion® solution was prepared by diluting a Nafion® 117 solution (Fluka) in ethanol.

The porous electrode was moistened with 0.1 % (V/V) solution of Nafion® in ethanol. On drying the electrode was coated at –500 mV with 50 ml of Hg coating solution at a flow rate of 6 ml/min. After coating the electrode was rinsed with 10 ml of the carrier electrolyte solution at a potential of –200 mV. During pauses, the cell was switched to a standby potential (Tab. 1). The electrode was used until fouling then it was replaced by a new one.

The samples were taken into amber glass bottles and stored in a refrigerator. The control measurements were performed at the same time as the chronopotentiometric measurements. Prior to analysis, the sample in the bottle was shaken and then let to sediment for few minutes. For analysis the supernatant was pipetted.

20 to 500 μL of the sample was added to 50 mL of 0.1 mol/L NaOH and on mixing the solution was immediately analysed. The waste water samples were analysed with the elaborated method independently in two laboratories by two different operators.

The accuracy of the results for waste water samples was checked independently in an accredited laboratory by the iodometric titration method according to the Slovak Standard STN 830530, part No. 31b (STN 83 0530-31, 1999). The method is based on the precipitation of sulphides as cadmium sulphides during a 24 h period, then cadmium sulphide is filtered off and the sulphide content was determined iodometrically.

2.3 Reagents and procedures for lead measurement

Nitric acid and hydrochloric acid were purified by subboiling and isopiestic distillation, respectively. Deionised and degassed water was used for preparing all solutions. All reagent solutions were checked for Pb by GF AAS before use.

Carrier electrolyte: 0.1 mol/L HCl.

Hg coating solution: 1 mg/L Hg(II) in 0.1 mol/L KSCN.

The bulk standard solution of 10 mg/L Pb^{2+} was prepared in the carrier electrolyte from a Certified Reference Material (1.000 g/L Pb, Merck® Darmstadt, Germany).

Coating of the porous electrode: The surface of the porous electrode E-53C (effective diameter 5 mm, length 3 mm, approximate void volume and surface 20 μL and 25 cm^2, respectively) was moistened with 20 µL of 0.1 % (V/V) solution of Nafion® in ethanol. On drying the electrode was coated at –500 mV with 50 mL of Hg coating solution at a flow rate of 3 mL/min. After coating the electrode was rinsed with 10 mL of the carrier electrolyte solution at a potential of –200 mV. The electrode was used until fouling thereafter it was replaced by a new one. To minimise electrode blocking with solid or colloidal particles, an in-line filter (0.45 µm pore size, material nylon) was used downstream of the cell.

Sampling and sample pre-treatment: The waste water samples were obtained from the sewage water treatment plant of an electroplating factory in North Slovakia. Complexing agents (not specified) and residual sulphides from the waste water treatment technology at various concentration levels were to be expected in the samples. The samples were collected into plastic bottles and immediately analysed. The samples were filtered before analysis and hydrochloric acid was added to a final concentration of 0.1 mol/L.

For the GF AAS measurements the filtered samples were diluted with water if appropriate and acidified with nitric acid so that its final concentration was about 0.01 mol/L.

2.4 Reagents and procedures for chromium measurement

Nitric acid and hydrochloric acid were purified by subboiling and isopiestic distillation, respectively. Deionised and degassed water was used for the preparation of all solutions.

Carrier electrolyte: 0.2 mol/L HCl and 0.001 mol/L EDTA . The bulk standard solution of 10 mg/L Cr(VI) was prepared in the carrier electrolyte from K_2CrO_4.

The bulk standard solution of 10 mg/L Cr(III) was prepared in the carrier electrolyte from a Certified Reference Material (1.000 g/L Cr(III), SMU Bratislava, Slovakia).

The porous electrode E-56, made of crushed glassy carbon particles (effective diameter of the electrode 5 mm, length 6 mm, approximate void volume and surface 40 µl and 50 cm^2, respectively) was activated by flushing with a 500 µg/L Cr(VI) solution in the carrier electrolyte at 800 mV for 5 min. After activating the electrode was rinsed with 10 ml of the carrier electrolyte solution at a potential of 700 mV. The electrode was used until fouling then it was replaced by a new one. To minimise electrode blocking with solid or colloidal particles, an in-line filter (0.45 µm pore size, material nylon) was used downstream of the cell.

The water samples were analysed immediately after sampling.

Cr(VI): To 10 ml of 1 mol/L HCl add 5 ml of 0.01 mol/L ethylenediamine tetraacetic acid (EDTA) solution. Adjust the volume to 50 ml with the sample and on mixing analyse the resulting solution.

Total Cr: To 50 ml of the sample add 1 ml of 1 mol/L HCl and 0.5 ml of 0.01 mol/L $KMnO_4$. Boil the solution gently until its volume reduces to about the half. On cooling transfer the solution to a 50 ml volumetric flask, add 10 ml of 1 mol/L HCl and 5 ml of 0.01 mol/L EDTA and adjust the volume to 50 ml with water.

The blank samples were prepared in the same way just pure water was used instead of the sample.

3. Sulphides in waste waters

Hydrogen sulphide is often present in waste waters especially in tanning processes. Oxidation of sulphide to sulphate must therefore be carried out prior to standard waste treatment processes. Various methods have been used for monitoring of sulphide concentrations such as titration, spectrometric, electrochemical and chromatographic techniques (Crompton, 1996). Mercury and mercury film electrodes have frequently been used for sulphide determination. Mercury coated platinum microelectrode was employed for in-situ determination of sulphides in water and sediment samples (Daniele et al., 2002).

Most of the above methods require the removal of sulphides from the interfering matrix prior to the measurement either through precipitation or evaporation which makes the procedures laborious and time consuming.

The presented procedure makes use of the formation of the negligibly soluble mercury sulphide on the electrode surface from the alkaline sample during the deposition step. In the next step the deposit is stripped into a slightly acidic electrolyte solution by applying a constant negative current, whereas the potential of the porous electrode is measured and evaluated. The main goal was to elaborate a simple, fast and reliable procedure not demanding a pre-separation step.

The low solubility of some sulphides facilitates the electrochemical determination of sulphides through stripping analysis by making use of an electrode material forming such a sulphide. Mercury and silver electrodes offer the best performance. However, silver electrodes, especially porous ones, have high background currents deteriorating their applications for low sulphide contents. Mercury and mercury coated electrodes are more suitable in this context, but are vulnerable to matrix interferences. It was found that the mercury coated bare porous electrode operated properly with waste water samples few hours only. Covering the surface with Nafion® prior to coating with mercury improved significantly its performance. The same electrode could be used for several days virtually without loss of the sensitivity. On deposition the cell is flushed with the carrier electrolyte consisting of sodium sulphate and acetate buffer at a pH of about 4.8 and the deposit is stripped through reducing the mercury sulphide to elemental mercury and hydrogen sulphide, the latter is washed from the electrode during the rinsing step. Porous electrodes possess a unique feature, absent in non-porous structures, namely there is a possibility to strip and deposit the analyte many times in a stopped flow regime. This can be used for signal accumulation in order to increase the signal to noise ratio (Beinrohr et al., 1994), or to shift the signal to a potential range with lower background level. In the case of sulphides it was found, that the background level is lower and signal reproducibility is higher if the stripping is made in the so called deposition-stripping-deposition-stripping sequence. Here, after deposition and rinsing with the carrier electrolyte the flow is stopped, the potential is shifted to a negative value (-600 mV for 10 s) causing the stripping (reduction) of HgS to Hg and HS^-, as well as the complete reduction of dissolved oxygen in the solution in the pores of the electrode. In the next step the potential is shifted again to a more positive value (0 mV for 30 s) where the HgS deposit is formed repeatedly from the HS^- ions in the electrode bulk. In the last step, this deposit is stripped again into the same but now the oxygen-free electrolyte by constant negative current and the stripping chronopotentiogram is measured. Owing to such in-situ reduction of oxygen there is no need for its preliminary removal, which makes the procedure simple and straightforward ([a] Manova et al., 2007).

The deposition potential influences significantly the signal response. Below –500 mV and above 100 mV no stripping signal was observed, the highest sensitivity was observed in the potential window of –350 mV to –50 mV. At potentials more negative than –500 mV no mercury sulphides is formed, at potentials above –50 mV sulphide is probably oxidised to sulphur, hence, no mercury sulphide is formed again.

For a sample volume of 1 mL taken for preconcentration the response is linear up to about 400-500 μg/L (regression data: slope 0.0095, intercept 0.017, correlation coefficient 0.9994) with a limit of detection and quantification (Mocak et al., 1997) of 1.6 μg/L and 5 μg/L, respectively. At a sample volume of 5 mL the detection limit was found to be about 0.5 μg/L.

The repeatability and reproducibility were evaluated from data obtained in a short measurement sequence of 15 analyses, and by repeating the measurement daily with a fresh sulphide solution of the same concentration (200 μg/L) during ten days, respectively. Hence, the repeatability and reproducibility were found to be 2.6 % and 4.8 %, respectively.

Owing to the large electrode surface, stripping current larger than –200 μA should be used. The measurement time at lower currents is longer than 5 min. The current of –500 μA ensured the best signal to noise ratio and fast measurement.

The waste water samples from a tannery contained dispersed colloidal substances and solids, so the key question was whether the sample preparation procedure (see Experimental) would ensure repeatable results. Sample volumes of 50 μL and 100 μL from the sedimented sample were pipetted to the 0.1 mol/L NaOH solution and analysed. The relative standard deviation of the results for these volumes was found to be 5.8 % and 2.4 %, respectively. Hence, the repeatability did not differed significantly from that for homogenous sulphide solutions. A typical signal of a diluted waste water sample is depicted in Fig. 3.

A possible interferent commonly present in waste waters from tanneries is sulphite. It was found that a 1000-fold excess of sulphites do not influence the signal of sulphide at all, and a 5000-fold excess causes a 8 % drop of the signal only.

The elaborated procedure was used for analyses of waste water samples taken from different locations of a tannery (Tab. 2). In most cases satisfactory agreement was found between the results obtained by the proposed procedure and the control method. However, in samples No 3 and No 6 the chronopotentiometric method provided much lower sulphide content than the control method. Theses samples spiked with known amounts of sulphides gave recoveries of the spikes near 100 %. Hence, the sample matrix virtually did not interfere. These samples, diluted with NaOH, were then heated in a microwave oven to a temperature of 80-95 °C and on cooling analysed again. The results obtained in such a way were significantly higher and correlated well with the control measurement (Tab. 2).

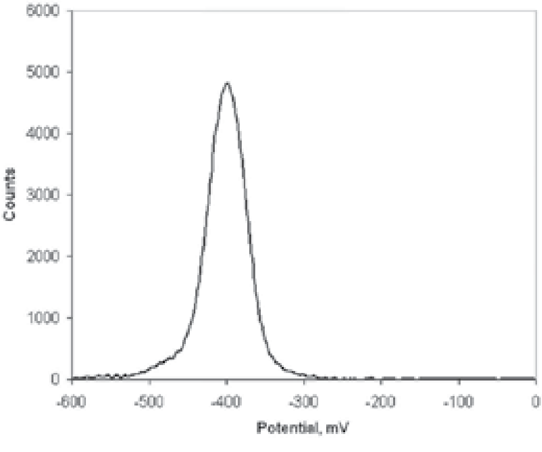

Fig. 3. Stripping signal of a real sample from a tannery containing 85 mg/L sulphide (50 μL of the sample diluted to 50 mL) ([a] Manova et al., 2007)

Sample	*Laboratory A mg/L*	*Laboratory B mg/L*	*Control analysis mg/L*
1	107.6 ± 4.6	95.1 ± 6.1 99.1 ± 7.2 [a]	97.3 ± 7.8
2	84.8 ± 10.8	89.3 ± 13.3 81.8 ± 9.2 [a]	76.1 ± 6.3
3	1.5 ± 0.3	3.5 ± 1.3 28.2 ± 1.8 [a]	27.5 ± 2.7
4	25.8 ± 3.2	21.1 ± 3.5 20.9 ± 2.9 [a]	28.5 ± 2.8
5	3.5 ± 0.4	3.9 ± 0.5 4.1 ± 0.6 [a]	4.7 ± 0.6
6	3.0 ± 0.5	2.9 ± 0.3 17.5 ± 1.5 [a]	16.5 ± 1.7
7	15.8 ± 2.4	17.8 ± 2.5 18.9 ± 3.5 [a]	18.5 ± 1.9
8	86.0 ± 1.7	85.6 ± 2.0 88.2 ± 2.5 [a]	91.3 ± 7.4
9	4.3 ± 1.8	3.0 ± 0.7 3.3 ± 1.1 [a]	3.15 ± 0.42

Table 2. Analyses of waste water samples from a tannery. [a] Sample heated prior to analysis ([a] Manova et al., 2007)

For the other samples, a heating did not influenced significantly the result. A plausible explanation is that the reference method evaluates also such sulphide species in some samples, which are not dissolved in the sodium hydroxide solution at laboratory temperatures.

4. Trace lead in waste waters

Electrochemical stripping techniques have been proved to be the most sensitive methods for some electroactive elements such as lead, cadmium, mercury and some others. In these techniques, the trace elements are deposited on a suitable electrode and then are stripped either potentiostatically, galvanostatically or chemically. The galvanostatic stripping (galvanostatic stripping chronopotentiometry) exerts some special features making this technique more suitable for routine use. The electronic control is simpler compared to voltammetric systems and the signal resolution is better due to smaller peak widths. However, the overlapping chronopotentiometric signals are not additive making signal deconvolution known in voltammetry virtually impossible.

Stripping analysis with matrix exchange can easily be performed by making use of a flow-through electrochemical cell (Bard & Faulkner, 2001; Stulik & Pacakova, 1987). Moreover, porous flow-through working electrodes may open the way to achieve complete electrochemical conversions both during the deposition and the stripping steps and hence to provide calibrationless analysis just by making use of the combined Faraday's laws for signal evaluation (Blaedel & Wang, 1979; Curran & Tougas, 1984). An alternative way to provide calibrationeless determination in stripping analysis is to deposit completely the analyte from a small sample volume to a vibrating working electrode (Jagner & Wang, 1995; Jagner et al., 1996).

Flow-through porous electrodes with pore size comparable to diffusion layer thickness possess some special advantages: high electrochemical yields, virtually up to 100 %, fast electrolysis in the electrode bulk, signal accumulation through repeated deposition-stripping cycles inside the electrode (Beinrohr et al., 1994), and direct coulometric titration in the pores of the electrode. However, owing to the large electrode surface, capacitive currents would deteriorate the measurement of low Faradayic currents. To compensate for this, electrode materials with low inherent background currents should be used and the background signal should be subtracted from the total signal.

Assuming an exhaustive deposition of the analyte at the working electrode during the deposition step, the transition time τ corresponding to the dissolution of the deposit during the stripping step is given by the Faraday's laws of electrolysis. Provided that the dissolution is done completely by the applied current, Eq. 1 and Eq. 2 can serve to calculate the analyte concentration directly from the analytical signal τ, i.e. the method is in principle calibrationless.

Trace concentrations of lead have usually been determined by AAS preferably making use of electrothermal atomisation. Lead can also be determined by electrochemical methods such as stripping voltammetry (Wang, 1985) and stripping chronopotentiometry. These methods exert excellent sensitivity, even better than GF AAS, robustness a low costs.

Lead can easily and virtually completely be deposited on mercury coated porous glassy carbon electrode ([b] Beinrohr et al., 1992). The lifetime of the mercury coating enhances significantly by pre-coating of the carbon surface by a thin layer of Nafion, which, additionally, makes the electrode surface more resistive against interfering organics. The lifetime of an electrode surface coated in such a way is at least one day and up to 100 measurements could be performed with the same coating.

The deposition of lead can be done either at a constant deposition potential or by using a constant deposition current. The former offers more selectivity the latter enhances robustness especially when analysing samples with significantly different matrices. Since the treated samples were similar in matrices, the potentiostatic pre-concentration mode was used in these experiments.

The sensitivity of the measurement is governed by two principal parameters: i) sample volume taken for analysis, and ii) stripping current. Obviously, the sensitivity increases by enhancing the sample volume and/or by decreasing the stripping current. The lowest sample volume taken for deposition is limited by an accepted precision of the sample injection with the applied instrument and is usually 0.3 – 0.5 mL. The largest sample volume is given by a reasonable duration of an analysis (5-10 min), hence being 5 – 15 mL. The stripping should be in the range of 50 to 400 μA, 100 μA was used in further experiments.

In diluted nitric acid media, the deposition efficiency was significantly lower then 100 %. Hence, the samples were acidified with hydrochloric acid, which ensures complete depositions.

The flow system enables a simple adjustment of the sample volume taken to the analysis and there is a possibility to match the parameters to samples with low and high lead contents simply by setting an adequate sample volume. The linearity of the method was evaluated by analysing a series of Pb solutions prepared in concentration range of 0.1 to 2000 μg/L. Obviously, the sample volume taken for pre-concentration should be matched to the expected concentration range.

The limit of detection (LOD) and limit of quantification (LOQ) values were calculated from the concentration dependence for the low concentration range (Fig. 4) and sample volume of 5 mL. The concentration dependence was evaluated by linear regression according to IUPAC recommendations (Mocak et al., 1997). The LOD and LOQ values were found to be 0.07 μg/L and 0.22 μg/L, respectively.

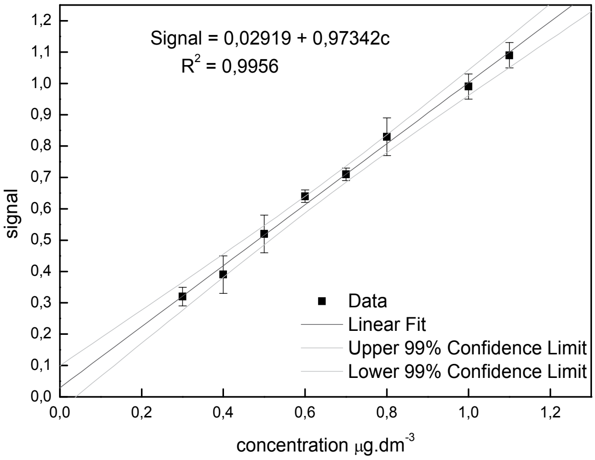

Fig. 4. Assessment of LOD and LOQ values. Sample volume 5 mL. Other parameters are listed in Tab. 1 (Strelec et al., 2007)

The repeatability and reproducibility were tested on the same standard solutions prepared once and stored in a 1000 mL volumetric flask at laboratory temperature, dark. The repeatability test was done with the same instrument by the same operator in a short sequence. The reproducibility test was carried out by different operators and in a daily pace. The repeatability and reproducibility for 11 measurements was found to be 1.1% and 3.7 %, respectively.

The accuracy of the method was checked by analysis of a certified reference material CRM 12-3-10 (SMU Bratislava, Slovakia; certified value for Pb and uncertainty: 0.029 mg/L and 0.006 mg/L, resp.). The elaborated method gave a value of (0.030 ± 0.001) mg/L.

Real water samples may contain various heavy metal ions, which could interfere. As Fig. 5 implies, only Cd affects the signal of lead, and that at concentration ratios higher than 1:50 (Pb:Cd), presumably owing to a coalescence of their signals (Fig. 6). The other tested species, Cu, As, Zn, Hg, Bi virtually do not interfere. Sn can only interfere in more concentrated hydrochloric acid solutions (over 0.5 mol/L), in the 0.1 mol/L HCl solutions used here its deposition is negligible.

The elaborated procedure was used for analyses of water samples from a water treatment plant of a galvaniser. The results are collected in Tab. 3 together with control data obtained by GF AAS. Notwithstanding the complex character of the sample matrix (salts, organics, sulphides, etc.), a reasonable correlation between the chronopotentiometric and AAS data was observed. The lower value found by chronopotentiometry in the sample No 3 may be attributed to the high concentration of complexing agents in this sample. This can partially be assigned to the high Pb contents in the samples enabling a higher dilution of the sample, which, favourably, minimises possible matrix interferences owing to the dilution of the matrix.

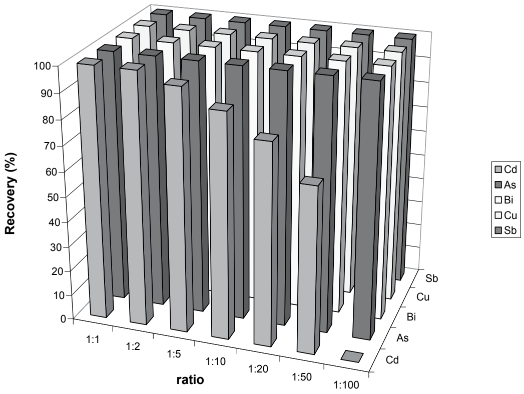

Fig. 5. Influence of some metal ions on the found values of Pb. Sample volume 5 mL, lead concentration 5 µg/L (Strelec et al., 2007)

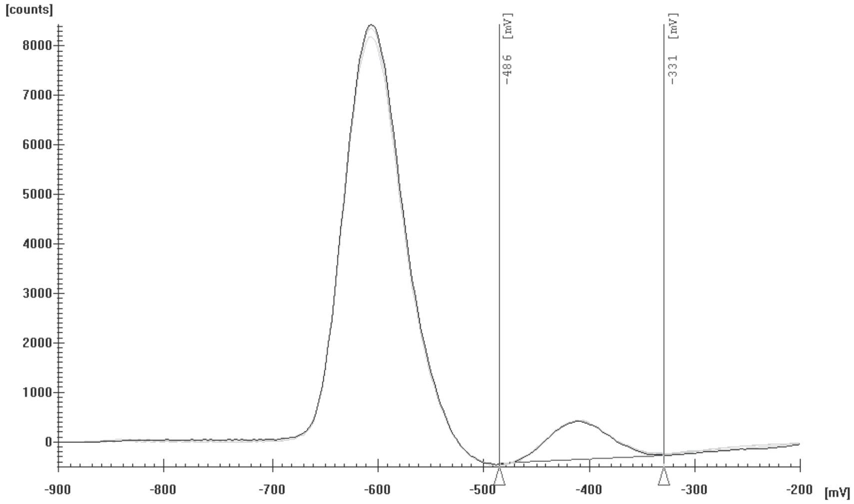

Fig. 6. Stripping chronopotentiogram of Pb (lower peak on the right) in the presence of an excess of Cd (left). Sample volume 5 mL, Pb and Cd concentrations 5 µg/L and 50 µg/L, resp. (Strelec et al., 2007)

Sample No	*Chronopotentiometry mg/L*	*GF AAS mg/L*
1	1.79 ± 0.03	1.75 ± 0.06
2	0.991 ± 0.011	0.989 ± 0.021
3	0.099 ± 0.005	0.118 ± 0.012
4	0.767 ± 0.012	0.794 ± 0.030
5	0.656 ± 0.025	0.661 ± 0.030
6	14.9 ± 0.2	14.7 ± 0.3

Table 3. Analysis of the waste water samples by flow-through chronopotentiometry and GF AAS. Results obtained from 5 repeated measurements (Strelec et al., 2007)

5. Chromium in water samples

Chromium is one of the most abundant elements on Earth. The amount of chromium in the environment has gradually been increased predominantly by industrial activities especially from tanneries, mines and incinerators. The toxicity of Cr(VI) to living organisms is well known and therefore a considerable interest has developed in its determination in environmental and industrial sites.

Chromium(VI) is usually determined by UV-VIS spectrophotometry by means of diphenylcarbazide in acidic solutions (Rudel & Terytze, 1999). Atomic absorption spectrometry with electrothermal atomisation (GF AAS) is one of the most sensitive methods for chromium determination in aquatic samples. Unfortunately, a direct measurement of Cr(VI) is not feasible with this and other atomic spectroscopic techniques. A separation of Cr species prior to the measurement is therefore inevitable either by means of a minicolumn (Cespon-Romero et al., 1996; Rao et al., 1998) or HPLC (Allen & Koropchak, 1993; Lintschinger et al., 1995; Andrle et al., 1997; Luo & Berndt, 1998). The procedure can be simplified by making use of a flow cell coupled in-line to a flame AAS instrument (Beinrohr et al., 1996), consisting of a porous electrode for oxidising Cr(III) to Cr(VI) and a sorbent for trapping Cr(VI). Cr(VI) is collected without electrolysis and on elution measured by AAS. Total Cr is measured after oxidising Cr(III) electrochemically to Cr(VI) which is collected together with the original Cr(VI) in the sorbent and then measured.

Electrochemical methods provide a simple tool for direct speciation of chromium. The most commonly used technique is based on adsorptive accumulation of the product of the reaction between Cr(VI) and diethyltriaminepentaacetic acid (DTPA) on the hanging mercury drop electrode. The adsorbed deposit is then cathodically stripped, mostly in the presence of nitrate giving rise to an intense catalytic current (Golimowski et al., 1985). Only Cr(VI) species give this product which enables the selective determination of chromate also in the presence of Cr(III). To bypass the use of the toxic mercury electrode, bismuth-film electrodes have recently been used (Chatzitheodorou et al., 2004; Lin et al., 2005).

In-electrode coulometric titrations facilitate the direct determination of Cr(VI) in water samples. The method makes use of a direct electrochemical reduction of chromate ions to Cr(III) in a porous glassy carbon electrode by constant current according to the electrode reaction given below:

$$CrO_4^{2-} + 8\,H^+ = Cr^{3+} + 4\,H_2O - 3\,e^- \qquad (3)$$

The potential of the electrode is monitored during the reduction indicating the end of the coulometric titration. The influences of experimental parameters, metrological figures and possible interferences will be investigated.

A new porous electrode exhibited low sensitivity which enhanced and stabilised gradually after few measurements of Cr(VI) samples. To avoid this initialisation period, the electrode was flushed with a Cr(VI) solution first and then the electrode was used for analyses. The lifetime of the electrode was limited by clogging the pores with solids and/or by a gradual and irreversible increase of the background signal due to a slow oxidation of the electrode surface. When an in-line filter was used to minimise electrode clogging, an average lifetime of an electrode was found to be at least three days, or several hundreds of measurements.

The value of the current forced to the electrode during a chronopotentiometric experiment affects significantly the sensitivity of the signal. In general, the lower the current the higher is the sensitivity, but the signal to background ratio is virtually not influenced. Theoretically, in a porous electrode with pore diameters near to the diffusion layer thickness an exhaustive electrolysis proceeds, the electrical charge consumed for the electrochemical change is given by the amount of the electrolysed species inside the pores of the electrode. The electrical charge consumed for the reduction of chromate is virtually independent on the value of the current used in the range of -10 to -1000 μA, being at the level of 40 μC and the function can be described by $y = 0.0004x + 39.454$ ($R^2 = 0.081$). However, the noise level enhances significantly at currents larger than -200 μA. Owing to the ohmic resistance of the solutions, the reduction peak is shifted to more negative potentials when enhancing the reduction current. At currents smaller than -10 μA the duration of a single measurement may exceed 5 – 10 min. Hence, reduction currents of –50 to -100 μA were used in further experiment ensuring low noise level and short measurement times.

The electrochemical reduction of chromate proceeds in acidic solutions. Nitric acid interferes (see below), in sulphuric acid media the reduction of frequently present Fe(III) totally coalesces with that for Cr(VI). Hence, hydrochloric acid was chosen as electrolyte for the reduction. Its content influences the peak position and especially the peak area. However, the sensitivity of the signal is not significantly improved at HCl concentrations above 0.2 mol/L, so such a concentration was used in further work.

The concentration range was tested up to several mg/L of Cr(VI) (Fig. 7). The response was found linear up to 500 μg/L. The lower concentration range was used for estimation of limits of detection and quantification (Table 4). The repeatability of the measurement was calculated from 10 measurements of Cr(VI) solutions with different concentrations in a short sequence. For reproducibility assessment, solutions the same concentrations were analysed in an interval of ten days.

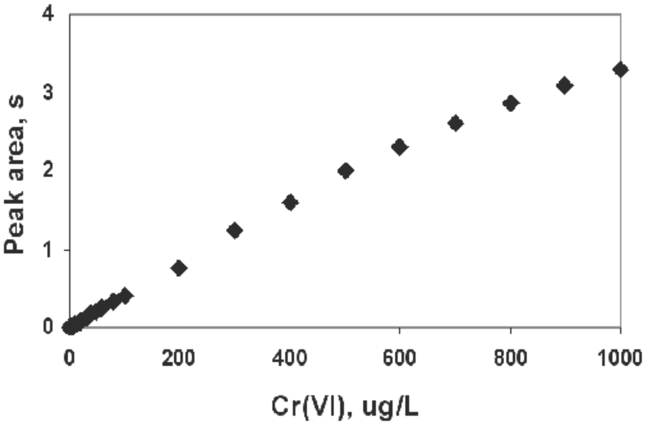

Fig. 7. Concentration dependence of the chromium signal. Reduction current –50 μA (b Manova et al., 2007)

Numerous substances may interfere in the reduction of chromate including species, which can be reduced at similar potentials, species adsorbing at the electrode surface or substances which would reduce chromate chemically prior to the measurement.

The influence of humic acids proved to be significant at contents higher than 0.5 – 1 mg/L (Fig. 8). The Cr(VI) signal decreases with increasing humic acid concentration which can be accounted for by chemical reduction of chromate to Cr(III) by humic substances in the acidic

Parameter	*Value*
Detection limit	1.9 µg/L
Limit of determination	6.0 µg/L
Linear range	(5 – 500) µg/L
Repeatability at 10 µg/L	5.9 %
100 µg/L	1.2 %
500 µg/L	0.6 %
Reproducibility at 10 µg/L	8.8 %
100 µg/L	1.8, %
500 µg/L	0.8, %
Measurement duration	3 min

Table 4. Analytical figures of merit for chromium determination ([b] Manova et al., 2007)

solution. As a proof of it, measurement of total Cr in the same sample solution gave the added content of Cr in the sample.

Iron(III), a common species in water samples interferes owing to its reduction at similar potentials as Cr(VI). This interference can partially be suppressed by addition of EDTA to the sample which forms a more stable complex with Fe(III) than that with Fe(II), shifting the reduction peak of Fe(III) to more negative potentials. The reduction peak of Cr(VI) is virtually not influenced by EDTA. The higher the EDTA concentration the larger is the shift of the Fe peak.

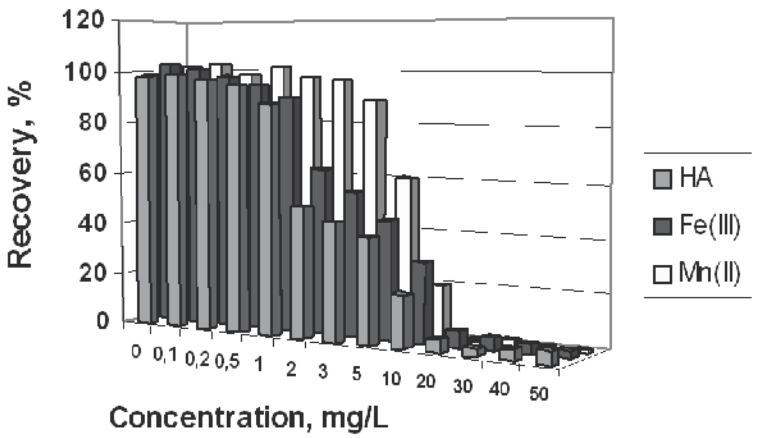

Fig. 8. Influence of humic acids (HA), Fe(III) and Mn(II) on the recovery of Cr(VI). Cr(VI) concentration 100 µg/L ([b] Manova et al., 2007)

Unfortunately, the concentration of EDTA is limited by its solubility in acidic solutions, here at concentrations above 0.001 mol/L, a white precipitate of EDTA was formed in the solutions after several hours. Nevertheless, in this way, Fe(III) concentrations up to 500 – 1000 µg/L can be tolerated. The interfering effect of higher Fe(III) concentrations can only be minimised by removing Fe(III) from the original sample, e.g. by making use of cation exchanger. Calcium and magnesium ions do not interfere.

Sulphate ions do not interfere. On the contrary, the signal of Cr(VI) decreased significantly with increasing concentration of nitrate ions or nitric acid above 0.1 mol/L. This can be assigned to a partial electrochemical reduction of nitric acid being in large excess which coalesces with the reduction peak of Cr(VI).

The influence of the tested surfactants is totally different. The neutral Triton X-100 virtually exerts no influence on the signal. Moreover, its addition to the solutions improves the reproducibility owing to easier removal of air bubbles from the flow system. The anionic sodium dodecylsulphate decreases the Cr(VI) signal already at concentrations above 3-5 mg/L. Sorption of the anionic surfactant on the positively charged electrode surface may be a plausible explanation. On the contrary, the cationic Hyamine enhances the signal significantly. In the presence of Hyamine is the coulombic content of the reduction peak much higher than expected from the Cr(VI) content in the porous electrode, so a sorption of Cr(VI) during the filling of the electrode with the sample seems to occur. However, the explanation of this phenomenon would need further investigation.

Total Cr can only be assessed with this method after oxidising Cr(III) to Cr(VI) and measuring it with the above procedure. The oxidation of Cr(III) with hydrogen peroxide in alkaline solution is simple and fast. However, on acidifying the resulting solution the formed Cr(VI) is immediately reduced back by the excess of hydrogen peroxide remaining in the solution after oxidation. Oxidation with $K_2S_2O_8$ in acidic media produced Cr(VI) as well but the excess persulphate interfered in the Cr(VI) measurement giving an intense reduction peak completely obscuring the Cr signal. The only applicable procedure found was the oxidation with permanganate in solutions acidified with HCl. The procedure is simple but time consuming owing to the need of a long boiling of the reaction mixture. The excess of permanganate is automatically removed by addition of EDTA after completing the oxidation.

The completeness of the oxidation and recovery were checked by means of synthetic samples in the concentration range of 10 to 500 μg dm^{-3}, as well as by means of real water samples spiked with known amounts of Cr. Satisfactory recoveries (90-110 %) were achieved in all cases.

Tap water, mineral water and river water samples were analysed by the elaborated procedure. The accuracy for total Cr content was checked by GF AAS measurements (Table 5). No values above the detection limit of the methods were found in these samples so the samples were spiked with Cr(VI) and Cr(III) for recovery tests. Recoveries about 100 % were obtained in all cases.

Sample	*Cr(VI) found μg/L*	*Total Cr found μg/L*	*Spike recovery %*	*Total Cr* [a] *μg/L*
Tap water	< 1.9	< 1.9		< 0.8
+ 50μg/L Cr(VI)	49.6 ± 1.9		99.2	46.7 ± 3.1
+ 50μg/L Cr(III)	< 1.9	49.8 ± 2.0	99.6	
Mineral water [b]	< 1.9	< 1.9		< 0.8
+50μg/L Cr(VI)	48.5 ± 2.1		97.0	47.9 ± 3.8
+50μg/L Cr(III)	< 1.9	51.5 ± 2.2	103	
River water [c]	< 1.9	< 1.9		< 0.8
+ 50μg/L Cr(VI)	51.0 ± 2.4		102	48.3 ± 4.1
+ 50μg/L Cr(III)	< 1.9	48.6 ± 2.8	97.2	

Table 5. Recovery test with water samples. a Found by GF AAS; b Mineral water "Miticka ticha" (Slovakia); c River L' Arve at Geneve, Switzerland (b Manova et al., 2007)

6. Conclusions

The stripping chronopotentiometric determination of sulphides provided reliable results for waste water samples from a tannery. The sample preparation is simple, even if in some cases the prepared sample should be heated prior to the measurement to obtain values corresponding with the control method. There is no need for a pre-separation step such as in titrimetric and photometric methods. The automatic on-line matrix exchange in the flow system after deposition minimises possible interferences from the sample matrix making this technique advantageous over the static batch ones. In general, the presented procedure allows the measurement of sulphide compounds, which form soluble sulphide in alkaline solution. It is evident, that different sample preparation procedures, e.g. boiling with mineral acids and distillation, may address some sulphide species not measured by this technique. The main advantages of the elaborated procedure are the simple sample preparation, no interference from dissolved oxygen and sample matrix, low detection limit, fast and full automatic measurement.

The determination of lead by flow-through chronopotentiometry proved to be a simple, sensitive, and accurate method for waste water analysis. The measurement, including sample preparation is fast, an average measurement cycle does not exceed 3-6 min. Due to the broad linear concentration range and low detection limit the method can successfully deal with samples with different lead concentrations.

The in-electrode coulometric titration of Cr(VI) in porous electrodes proved to deliver reproducible and accurate results in a concentration range of about 5 to 500 μg/L. Owing to the preconcentration effect the detection limits of the adsorptive stripping methods are much lower (Table 1) but the presented procedure offers some significant advantages. The limit of detection enables to use the method for assessment of chromium content in drinking water below the threshold value (50 μg/L). The sample preparation for Cr(VI) determination is simple and fast and dissolved oxygen need not to be removed. Owing to the long lifetime of the porous carbon electrode, the procedure is also suitable for field applications and long-term unattended measurements, e.g. in process systems. Compared to photometric methods for Cr(VI), coloured species do not interfere. However, total Cr can only be assessed after a chemical oxidation of Cr(III) to chromate. Colloidal and solid particles in the solutions may clog the pores of the electrode and therefore must be removed. High contents of organic substances in some waste waters may interfere and the elimination of such interferences is the main goal in the improvement of the presented procedure.

The intrinsic simplicity of chronopotentiometric measurements, especially in flow-through mode has made this method a suitable tool for routine and on-line control of electrochemically active species in environment, waste water treatment and chemical technologies. Heavy metals, some anions and simple compounds can be targeted by the technique. Obviously, the direct contact of the sensor (electrode) with the sample may be a source of various interferences not only due to possible presence of electrochemically similar species but predominantly due to electrode failure caused by sorption and deposition of matrix components on the electrode surface. Yet, proper sample pre-treatment and adequate experimental parameters minimize or even completely eliminate these effects. As a consequence, this measurement principle is becoming preferred now predominantly in industrial applications for unattended on-line monitoring purposes.

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5

The Chemical Oxygen Demand Modelling Based on a Dynamic Structure Neural Network

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1. Introduction

Wastewater treatment process aims at achieve the purpose of purification by degradation of organic matter in water. To ensure the effluent water quality, some indicators should be measured, including chemical oxygen demand (COD), Biochemical oxygen demand (BOD), etc. Through the prediction on effluent indicators can provide effective guidance for the operation of wastewater treatment plant.

Wastewater treatment process itself is a nonlinear, time-delay process with complex reactions. Thus, when using traditional mathematical model there is often a lack of accuracy, large amount of calculation and lack of flexibility in system simulation, while the prediction based on neural network model can effectively eliminate these disadvantages because of its learning mechanism. Nowadays, applying neural network in wastewater treatment process has become a research hotpot, and some breakthroughs were achieved in terms of algorithms or modelling.

zhu, et al. used MLP model to reduce the data dimension, then used the time-delay neural network to predict the effluent BOD online. Chang, et al. reduced data dimension through principal component analysis(PCA), then used extracted system inherent characteristics from data by fuzzy C clustering, at last, TSK-type fuzzy inference system was used to predict the effluent COD. Chai et al. proposed a activated sludge process mechanism model based on hierarchical neural network, connecting the mechanism model and neural network in cascade way and with the neural network identifying the reaction rate of nonlinear components in activated sludge process model to predict the effluent COD.

The above evidences show that artificial neural networks can directly establish the model according to the input / output data without prior knowledge of the condition object, and has strong online correction ability. For the process with a large amount of data information which can not be described by rules or formulas, the artificial neural network shows great flexibility and adaptability which is ideal for wastewater treatment systems. However, these network models have the same shortcomings that the network structure would no longer able to modify after finalized in the early stage of designing. For the different cases of wastewater treatment process, the re-design of neural network prediction model is necessary. To solve this problem, meet the needs of the object by dynamically adjusting the neural network structure is an available approach.

Huang et al. proposed a simple sequential learning algorithm called the "RBF growing and pruning algorithm" (GAP-RBF), which was later developed into the GGAP-RBF algorithm.

The GAP-RBF and GGAP-RBF methods use the "significance" of a hidden node to decide whether to add new nodes or reduce the number of redundant nodes. The "significance" of the node is also linked to the learning accuracy. However, since these algorithms are on-line procedures, they do not optimize the network over all past training data. Moreover, network initialization of the GAP-RBF algorithm requires a priori knowledge of the data which may not be available.

This chapter presented a repair algorithm for the design of a Radial Basis Function (RBF) neural network. The proposed repair RBF (RRBF) algorithm starts from a single prototype randomly initialized in the feature space. The algorithm has two main phases: an architecture learning phase and a parameter adjustment phase. The architecture learning phase uses a repair strategy based on a sensitivity analysis (SA) of the network's output to judge when and where hidden nodes should be added to the network. New nodes are added to repair the architecture when the prototype does not meet the requirements. The parameter adjustment phase uses an adjustment strategy where the capabilities of the network are improved by modifying all the weights. The algorithm is shown to be effective by approximating a non-linear function, so it is used to model the key parameter, chemical oxygen demand (COD) in the waste water treatment process. The results of simulation show that the algorithm provides an efficient solution to problems.

The chapter is organized as follows. Section2 introduces the methods and problems of the modelling the key parameter, chemical oxygen demand (COD) in the waste water treatment process, and gives the methods of measuring COD. Section3 gives a short description of the RBF neural network and the SA output model; briefly analyses the repair method which is used to add hidden nodes and describes the algorithm which adjusts the parameters; the proposed method is benchmarked against some well-known dynamic RBF algorithms. In order to demonstrate the superior performance of the proposed RRBF neural network, the algorithm is applied to approximating a nonlinear function. Section4 finishes the Soft measurement technique for COD in the waste water treatment process. The conclusion and Future work are given in Sec. 5.

2. Wastewater treatment process

2.1 The problem in COD measurement

Wastewater treatment plants are complex nonlinear systems, subject to large disturbances, where different physical (such as settling) and biological phenomena are taking place. Many models have been proposed in the literature for wastewater treatment process but their evaluation and comparison are difficult. To ensure the good condition and effluent quality during wastewater treatment operation process, the key parameters should be obtained in time. On the one hand, wastewater treatment aims on reducing the environmental pollution, which requires detecting the effluent COD, BOD, TN, TP etc. according to related national effluent standard; on the other hand, the normal operation and control implementation of each wastewater treatment link depends on real-time detection of controlled variables. Among these effluent parameters, COD, which indirectly represents the water organic pollution degree by DO consumption through microbiology metabolism, is an important index accords with the practical self-purification situation and the routes of most wastewater treatment processes. Therefore, the detection of COD is significant while some inevitable defects exist by using conventional approaches like COD on-line analyzer: the process of COD is complicated, the time spent on detecting will greatly lags to the operating time in practice and the results can't reflect the real situation in time [1]; COD on-line analyzer is too expensive to extend. Thus, as a cost-effective tool for replacing expensive on-line sensor, the research on soft-sensing became active since 1990s. Combining soft-sensing with neural network to predict key parameters and guide, neural networks are wildly applied in process modelling and prediction. Chang, et al. reduced data dimension through principal component analysis(PCA), then used extracted system inherent characteristics from data by fuzzy C clustering, at last, TSK-type fuzzy inference system was used to predict the effluent COD. In this section, the soft-sensing technology applied in wastewater treatment process will be introduced.

2.2 Soft-sensing technology

Soft sensors have been reported to supplement online instrument measurements for process monitoring and control. Both model-based and data-driven soft sensors have been developed (B. Lin et al., 2007). As solution for the above problems, the core of soft-sensing is mathematical modelling for the objects. To obtain optimal estimation for primary variables and set up a soft-sensing model which is suitable for wastewater treatment process, selecting appropriate instrumental variables according to wastewater treatment characteristics is a must. Principal component analysis can reduce the dimension and correlation of process variables (W. Ran et al., 2004), data preprocessing can obtain the correct data. So the design steps for soft-sensing are as follows:

a. Data preprocessing

Influenced by precision and reliability of measuring instrument and measuring environment, the measuring errors are inevitable. Firstly, the ones with different magnitude from others were deleted. Then the measuring samples should be handled by data normalization using the following:

$$p_{ij}^{*} = \frac{p_{ij} - \overline{p}_j}{s_{jj}} \tag{1}$$

Where i is the number of samples, j is the component of samples, p_{ij} is the j th component of the i th sample, $\overline{p}_j$ is the mean of the j th component of the samples, S_{jj} is the standard error of the variable p_j.

b. Principal component analysis

Wastewater treatment process is complicated, contains many variables. And there exists quite linear correlation among those measuring variables and data.

Create a matrix $p = [p_1, p_2, \cdots, p_m]$, which composed of process variables and divided by columns, to calculate its covariance matrix S. The characteristic roots of S are listed as $\lambda_1 \ge \lambda_2 \ge \cdots \lambda_m \ge 0$, of which the corresponding unit orthogonal Eigenvectors are composed of matrix $L = [L_1, L_2, \cdots, L_m]$ (Load Matrix) Dividing x into the exterior product of principal components' sub-matrix T and Load Matrix L, added residual term E, as follows:

$$x = TL^T + E = T_1 L_1{}^T + T_2 L_2{}^T + \cdots + T_n L_n{}^T + E$$

Then calculate m (minimum required number of principal components) which make cumulative variance contribution rate $\eta = \sum_{i=1}^{n} \lambda_i / \sum_{j=1}^{m} \lambda_j > 90\%$, select relevant n principal components in T. Under the precondition $\eta > 90\%$, four instrumental variables which mostly influenced effluent COD were chosen by analyzing the relation between principal components and instrumental variables, they are SS, pH, oil and NH_3-N.

c. Establishment of soft-sensing model

The well-known mathematical modelling and neural networks methods have limitations to incorporate the key process characteristics at the wastewater treatment plants which are complex, non-stationary, temporal correlation, and nonlinear systems (M. H. Kim et al., 2009). To build soft-sensing model of key water quality parameters, some systematic methods of neural networks modelling. Wang, W. (W. Wang & M. Ren, 2002) used BP neural network to predict BOD and COD etc. key parameters by modelling wastewater process; Guclu, D (D. Guclu & S. Dursun, 2008) used an artificial neural network to implement the prediction of effluent COD concentrations. The common characteristic of these techniques is that the feed forward neural network was used to model the wastewater treatment process (J. B. Zhu et al., 1998). Used MLP model to reduce the data dimension, then used the time-delay neural network to predict the effluent BOD online. Chai et al. (T.Y. Chai et al., 2009) proposed a activated sludge process mechanism model based on hierarchical neural network, connecting the mechanism model and neural network in cascade way and with the neural network identifying the reaction rate of nonlinear components in activated sludge process model to predict the effluent COD. Chen et al. (Q. L. Chen et al., 2010) proposed a recurrent neural network model to identifying the BOD by modelling wastewater process.

Researches show that artificial neural network is able to model the wastewater treatment process. However, wastewater treatment process is a highly nonlinear and state-varying dynamic process; the network's dynamic performance will vary according to different network structure because of the single and immutable mapping ability of fixed-structure neural network. Therefore, a self-constructing neural network (J. F. Qiao & H. G. Hang, 2010) which combined RBF neural networks and principal component analysis technology will be presented in section 3. The result of principal component analysis efficiently included in the key modelling information of the wastewater treatment process. The above four variables are used as the input of RBF model while effluent COD as the output, then we choose the proper number of neurons in the hidden layer and train the network by learning algorithm. This neural network would adjust network structure according to the complexity of wastewater treatment process, which would significantly improve the soft sensor's performance.

3. A Self-constructing RBF neural network

This section will introduce a self-constructing RBF neural network which based on a repair algorithm. Cell replacement therapy is emerging as a novel method for restoration of the defective tissues by repairing the inactive cells. This innovative strategy has attracted considerable attention to the human embryonic stem cell recently (Mathur A et al., 2004). Similarly, it is well known that the connecting cells of the biological networks are repaired in the neural systems (Noriaki Suetake & Eiji Uchino, 2007). A Radial Basis Function (RBF) neural network is a simple neural system model, which is often applied to machine learning problems, because it can approximate non-linear mappings directly from input patterns (S.S. Panda et al., 2008; Xabier Barandiaran & Alvaro Moreno, 2008). In theory, all RBF network topologies should be able to learn any given task to some level of competency. In reality, however, a given topology can be both a bottleneck and a constraint on a system. If the size of the network is chosen incorrectly it becomes moribund, rendering the results meaning less (A. Esposito et al., 2000; Wang, X. X. et al., 2004). Consequently a lot of research has focused on the difficult problem of determining the optimal size of a RBF neural network (S. Chen et al., 1991 & 1996). The size of the input and output layers in a RBF neural network is fixed – only the size of the hidden layer can be modified. Two main methods, "growing" and "pruning", have been developed to dynamically change the size of a network. Esposito et al. (A. Esposito et al., 2000) have proposed a growing method based on an evolutionary optimization strategy. However, this method has a number of drawbacks: it requires a large amount of processing power; the convergence time is very long, and the convergence is sometimes premature. To reduce the amount of computational time an a priori clustering method has been proposed (X. X. Wang et al., 2004). Unfortunately the proposed algorithm is intrinsically flawed limiting its usefulness (N. Y. Liang&G. B. Huang, 2008). Orr (M. J. L. Orr, 1995) has proposed a regularized forward selection (RFS) algorithm, based on subset selection (S. Chen et al., 1996), which combines forward subset selection and zero-order regularization. However, the subset selection method has several major disadvantages, the worst of which is that in order to increase the chance of obtaining a satisfactory RBF network; it has to use a very large set of candidate RBF nodes with different centers and radiuses. There are a number of other growing strategies (K. Li, J. Peng & G. W. Irwin, 2005; A. L. I. Oliveira et al., 2006 ; J. Gonzalez et al., 2003), but in all these methods the criterion for determining growth suffers from a lack of objectivity. Many of them are also time-consuming, sensitive to the input data, and do not consider the effect of the RBF output. Yingwei et al. (L. Yingwei et al., 1998) have proposed a pruning strategy, based on the relative contribution of each hidden node to the overall network output, which aims to reduce the complexity of the RBF neural network. In theory the size of the final neural network obtained by this method is minimal. Other methods for pruning RBF neural networks have been proposed by Salmer′ on et al., (M. Salmer′ on et al., 2001) Rojas et al., (I. Rojas et al.,2002) and Hao et al. (P. Hao & J. Chiang, 2006). A major problem with pruning methods is that they often require more computational time than growing methods. In fact, there are also quite a large number of parameters or variables that need to be preset; the training data needs to be stored and re-used for pruning purposes. A promising alternative is to combine growing and pruning methods together. Huang et al. (G. B. Huang et al., 2004) proposed a simple sequential learning algorithm called the "RBF growing and pruning algorithm"(GAP-RBF), which was later developed into the GGAP-RBF algorithm (G. B. Huang et al., 2004; Q. Meng & M. Lee, 2008). The GAP-RBF and GGAP-RBF methods use the "significance" of a hidden node to decide whether to add new nodes or reduce the number of redundant nodes. The "significance" of the node is also linked to the learning accuracy. However, since these algorithms are on-line procedures, they do not optimize the network over all past training data. Moreover, network initialization of the GAP-RBF algorithm requires a priori knowledge of the data which may not be available. Lian et al. (J. M. Lian et al., 2008) have proposed a self-organizing RBF neural network (SORBF) for real-time approximation of continuous-time dynamic systems. The aim of the SORBF network is to develop an algorithm that can be used in real time processes. However, the authors do not investigate the failure cases for the algorithm. How to choose a suitable criterion to design RBF neural network architecture remains an open question.

Section 3 presents a new RBF neural network design method which is called the "repair RBF neural network algorithm" (RRBF). RRBF performs simultaneous network architecture design and parameter optimization within an integrated analytic framework. This approach has two technical advantages. The first advantage is that the method is not dependent on the input data: when the sensitivity analysis (SA) of the RBF output indicates that the prototype is not suitable, the RBF architecture is repaired. The second advantage is that the criterion used to determine whether the network should be repaired or not is more objective than the criterion used in other similar time-based methods (e.g. RFS and GAF-RBF): it is based on the sensitivity analysis (SA) value which calculates the contribution from hidden nodes over a given time period (t + 1, t + 2, . . . , t + m, where m is the period). The learning process starts by randomly initializing a single prototype in the feature space; then, the prototypes undergoes adaptive repair until the most appropriate number of prototypes is reached.

3.1 Previous and related works

a. RBF neural network

A standard RBF neural network consists of three layers: an input layer, a hidden layer, and an output layer. Fig. 1 shows a schematic diagram of the RBF network. As the nodes in the input layer represent the variables from input space and the nodes in the outer layer represent the desired response, the number of nodes in the input and output layers is configured in advance. A learning algorithm uses the defined optimization criteria to minimize the error between the actual response and the desired response.

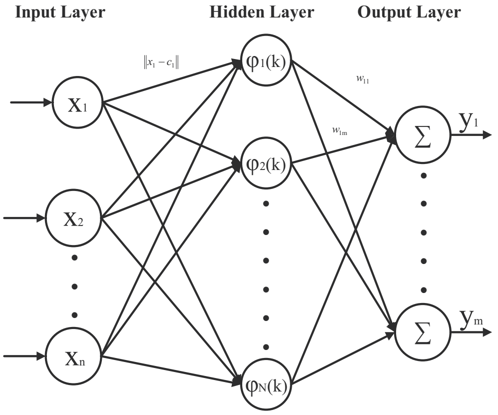

Fig. 1. Schematic diagram of RBF neural network (RBFNN)

As depicted in Fig. 1, the r -th output node of the RBF network can be expressed as follows:

$$y_i = \sum_{k=1}^{p} \phi_k(\|x - c_k\|) \cdot w_{ik},\ i = 1,2,\cdots,m \tag{2}$$

where $x = [x_1, x_2, \cdots, x_n]^T$ is an input value; n is the number of input node; c_k is the k -th center node in the hidden layer, $k = 1,2,\cdots,p$, and p is the number of hidden nodes;

$\|x - c_k\|$ denotes the Euclidean distance between c_k and x; $\phi_k(\bullet)$ is the nonlinear transfer function of the *k*-th center; w_{ik} is the weighting value between the *k*-th center and the *i*-th output node; and m is the number of output nodes.

Equation (2) reveals that the output of the network is computed as a weighted sum of the hidden layer outputs. The nonlinear output of the hidden layer are described as $\phi_k(\bullet)$, which are radial symmetrical. Here the function chosen for this neural network is Gaussian function, and the description is shown as follows:

$$\phi(x) = e^{(-\frac{(x-v)^2}{\delta^2})} \tag{3}$$

where v and δ are the parameters of position and width of the centers. The activation functions commonly used for the classification and regression problems are in the Gaussian functions because they are continuous and differentiable; they provide softer output and improve interpolation capabilities. The significant parameters to design an RBFNN for solving problems are shown as:

1. The RBF position of the centers v;
2. The width δ of the centers;
3. The weights w;
4. The number of the hidden nodes p;

Based on 1), 2) and 4), the initializations of the centers are very important, if an incorrect initialization of the centers is performed, the approximation error could be increased. The reason is that during the execution of a local search algorithm to make a fine tuning of the centers and the radius, there is a possibility of falling into a bad local minimum. An on-line self-organizing algorithm is used for selecting the centers of the RBFNN in this chapter. This algorithm can add new centers to repair the RBFNN, which solves the pre-set problem of the conventional RBFNN. Meanwhile, the width of the centers is very important, if the width is not appropriate for the RBF, the training time will be heavy. When the RBFNN selects correct centers, the parameters of the widths and the weights will be adjusted at the same time.

b. The Sensitivity Analysis of Model Output (SAMO)

A thorough description of sensitivity analysis methods can be found in (Andrea Saltelli et al., 2006). The most common SA is sampling-based. There are several steps to conduct SA. The following steps can be identified as (the details can be found in (Andrea Saltelli et al., 2006)):

Step 1. Define the model, its input factors and output variable.
Step 2. Assign probability density functions or ranges of the variation to each input factor.
Step 3. Generate an input matrix through sampling design.
Step 4. Evaluate the output.
Step 5. Assess the influences or relative importance of each input factor on the output variable.

At Step 4), an empirical probability distribution for the output can be created which may lead to a first step of uncertainty analysis. After quantifying the variation of the output, SA consists in apportioning the variance of the output according to the input factors. The representation of the results can be described as the contribution to the input that describes the variance of the output into the percentages that each factor is accounting for. In this way, the variance decomposition may allow the identification of the most influential factors. Then the SA should present analyses over the full range of plausible values of key parameters and their interactions, to assess how the change in response impacts changes in key parameters.

Sensitivity analysis (SA) is an available tool (J. Cariboni et al., 2007; A. Saltelli et al., 2000) which may be used to study the behavior of a system, or a model, and to ascertain the contribution ratio of the outputs depending on each or some of the input parameters. Among the SA methods, quite often they are identified almost as a mathematical definition, with a differentiation of the output respecting to the input. For this reason, quantitative measure of sensitivity, such as the EFAST method (A. Saltelli et al., 1999) is described as follows:

$$S_h = \frac{Var_h[E(Y|Z_h = \alpha_h)]}{Var(Y)} \tag{4}$$

where, Z_h denotes an input factor, $h = 1,2,\cdots,p$, and Z_h represents the output connecting value of the hidden nodes in the RBFNN in this chapter. Y is the model response, $E(Y|Z_h = \alpha_h)$ is the expectation of Y conditional on a fixed value α_h of Z_h and the variance Var_h is taken over all the possible value of Z_h. The ratio S_h represents the main effect. It is called the first-order index in the SA terminology. Thus, the main effect of a factor represents the average effect of that factor on the response or conversely these methods allow the computation of that fraction of the variance of a given model output which is due to each input factor. In addition to the computation of the EFAST method which also provided an estimation of the total sensitivity index ST_h. The total effect includes the main effect as well as all the interaction terms involving that factor. The total effect is defined by:

$$ST_h = \frac{Amount\ of\ \mathrm{mod}\,el\ response\ \mathrm{var}\,iance\ \ Z_h}{Model\ response\ \mathrm{var}\,iance} \tag{5}$$

The model is additive when the response is nonlinear but interactions are negligible. In that case, the main effects are the suitable indexes for the sensitivity analysis of model output (Philippe Lauret et al., 2006).

EFAST is based on the Fourier decomposition of the variance in the frequency domain. This method is especially suited for a quantitative model independent global SA. The computational cost of this method is the number of model evaluations required and is a function of the number of input factors and the complexity of the model. The ever-increasing power of computers tends to make these global methods affordable for a large class of models. Among the SA methods, the total sensitivity index is undoubtedly the best guide to quantitatively rank the factors by order of importance. Indeed, even if this occurs rarely, interaction effects on a model response may dominate the main effects. So, whether the interaction effects are taken into account or not, the analysis may result in a different ranking of the factors' importance.

3.2 The repair method for selecting hidden nodes of RBFNN

Considering the intrinsic structure of RBFNN, if the RBFNN consists of assured inputs, certain hidden nodes and outputs, it can only adjust the weights v , δ and w which are in the hidden layer or connecting the hidden nodes and the output nodes. In this chapter, the single response relationship between the hidden neurons and the output of the RBF is discussed. We state that the relevance of a hidden node is related to its influence on the RBF response. This is the key idea of the method proposed to determine the optimal architecture for the RBF. The parameters w of the hidden nodes are the input values of the repair algorithm based on the sensitivity analysis (SA). And this SA is based on the Fourier decomposition of the variance in the frequency domain.

a. Selecting Hidden Nodes

A generic model is assumed to describe an RBF neural network system. The model is represented by a mapping f (a deterministic or stochastic function) which relates the inputs domain to the output space:

$$Y = f(Z_1, Z_2, \cdots, Z_p) = \sum_{i=1}^{p} \beta_i Z_i \tag{6}$$

The input factors $(Z_1, Z_2, \cdots, Z_p)$ are supposed to be the variables described by the parameters w of the RBF hidden nodes, $Z_1 = w_{1\bullet}, Z_2 = w_{2\bullet}, \cdots, Z_p = w_{p\bullet}$, p is the number of the nodes in the output layer; $w_{1\bullet} = [w_{11}, w_{12}, \cdots, w_{1m}]^T$, m is the number of the nodes in the output layer. Y is taken to be a scalar even in the application we shall consider each output variable in turn. Based on the EFAST method, the polynomial expansion can be described again. The range of the factor Z_h is $\left[a_q, b_q\right]$, so the Z_h performances as follows:

$$Z_h^{(q)} = (b_q + a_q/2) + (b_q - a_q/2)\sin(\omega_h s^{(q)}) \tag{7}$$

where, $s^{(q)} = 2\pi n/N$, ω_h is the frequency, q is the simulation number, N is the total simulation number. If $Z_h^{(q)}$ is straightforward to note $z_h^{(q)} = \sin(\omega_h s^{(q)})$ and that the formula (6) should be as:

$$\begin{aligned} Y^{(n)} = Y_0 &+ \sum_{i=1}^{p} \beta_i \sin(\omega_i s^{(q)}) \\ &+ \sum_{i=1}^{p}\sum_{j=1}^{p} \beta_{ij} \sin(\omega_i s^{(q)}) \sin(\omega_j s^{(q)}) \\ &+ \cdots \end{aligned} \tag{8}$$

Based on this formula, the linear effect of Z_h corresponds to the Fourier amplitude at the fundamental frequency ω_h. In EFAST, each input factor Z_h is related to a frequency ω_h and a set of suitable defined parametric equations:

$$Z_h(s) = G_h(\sin(\omega_h s)) \qquad h = 1, 2, \cdots, p \tag{9}$$

The equations allow each factor to vary in a given range, as the new parameter s is varied. They define a curve which explores the input factors' space systematically. As s varies, all the factors oscillate at the corresponding driving frequency ω_h and their range is systematically explored. For the EFAST method, a parametric representation of the form is often used.

$$Z_h(s) = \frac{1}{2} + \frac{1}{\pi}\arcsin(\sin(\omega_h s)) \tag{10}$$

This transformation allows a better coverage of the factors' apace since it generates samples that are uniformly distributed in the range [0, 1].
If the range of variation of the factor Z_h is $[\,a,b\,]$, the parametric representation of the form should be:

$$Z_h(s) = \frac{b+a}{2} + \frac{b-a}{\pi}\arcsin(\sin(\omega_h s)) \tag{11}$$

As each factor Z_h oscillates periodically between $[\,a,b\,]$ at the corresponding frequency ω_h, the model output Y exhibits different periodicities that result from the combination of the different frequencies $\omega_{i=1,\cdots,p}$, whatever the model f is. Just for Fourier amplitudes, the p-factor model can be described as follows:

$$f(s) = f(Z_1(s), Z_2(s), \cdots, Z_p(s)) \tag{12}$$

where, the range of s is $[-\pi, \pi\,]$, so the expanded in a Fourier series of the form:

$$f(s) = \sum_{j=-\infty}^{\infty} (A_j \cos(\omega_j s) + B_j \sin(\omega_j s)) \tag{13}$$

where, the Fourier coefficients are defined as:

$$A_j = \frac{1}{2\pi}\int_{-\pi}^{\pi} f(s)\cos(\omega_j s)ds\,,\; B_j = \frac{1}{2\pi}\int_{-\pi}^{\pi} f(s)\sin(\omega_j s)ds\,;\text{ the range of } s \text{ is } [-\pi,\pi\,].$$

Therefore, N equally spaced sample points are required to perform the Fourier analysis. N represents the sample size and coincides with the number of model evaluations. Based on the Fourier translation, the variance D_y can be computed as:

$$D_y = Var(Y) = 2\sum_{k=1}^{+\infty}(A_k^2 + B_k^2) \tag{14}$$

The portion of the variance of Y explained by Z_h alone is:

$$D_y = Var_{Z_h}[E(Y|Z_h)] = 2\sum_{k=1}^{+\infty}(A_{k\omega_h}^2 + B_{k\omega_h}^2) \tag{15}$$

where, $A_{k\omega_h}$ and $B_{k\omega_h}$ denote the Fourier coefficients for the fundamental frequency and its higher harmonics $k\omega_h$. Then the expansion of the main effect is given by:

$$S_h = \frac{Var_{Z_h}[E(Y|Z_h)]}{Var(Y)} = \frac{2\sum_{k=1}^{+\infty} A_{k\omega_h}^2 + B_{k\omega_h}^2}{Var(Y)} \tag{16}$$

We stated above that in order to evaluate the main effect of Z_h, one must calculate the Fourier coefficients at the fundamental frequency ω_h and all the harmonics.
Because there is M interference factor (usually set to 4 or 6 in the SA community). So that the first-order sensitivity index is approximated by:

$$S_h = \frac{Var_{Z_h}[E(Y|Z_h)]}{Var(Y)} = \frac{2\sum_{k=1}^{M} A_{k\omega_h}^2 + B_{k\omega_h}^2}{Var(Y)} \tag{17}$$

The number of simulation runs represents the sampling frequency and, to meet the Nyquist criterion, it must equal to:

$$N = 2M\omega_{\max} + 1, \; (\omega_{\max} = \max(\omega_i)) \tag{18}$$

The variance $Var(Y)$ can be evaluated in the frequency domain through the following relationship:

$$Var(Y) = 2\sum_{w=1}^{\frac{(N-1)}{2}} (A_\omega^2 + B_\omega^2) \tag{19}$$

A_ω and B_ω denote the Fourier coefficients at frequency ω. In fact, based on this analysis the total sensitivity index ST_h can be shown as:

$$ST_h = \frac{\sum\limits_{\omega=M\max(\omega_h)+1}^{\frac{(N-1)}{2}} (A_\omega^2 + B_\omega^2)}{\sum\limits_{\omega=1}^{\frac{(N-1)}{2}} (A_\omega^2 + B_\omega^2)} \tag{20}$$

The advantages of the ST_h make the judgments in this chapter much better than the S_h. Based on the former analysis of ST_h, the main steps of the proposed approach for selecting hidden nodes in the RBF are as table 1. For the proposed total sensitivity index, some points have to be highlighted. First, usually, the sensitivity index for the ratio of the inputs is dependent on the current time ($t+m$). But the total sensitivity index can calculate the contribution of period of time ($t, t+1, \cdots, t+m$) which is more objective then the others based on the current time ($t+m$). Second, the computation of total sensitivity index occurs when the FNN has been trained into a minimum of the error function or when over fitting begins. But this proposed approach is not necessary. In other words, computation of total sensitivity index starts when the FNN has been trained for some epochs in this chapter.

b. Adding New Nodes

As the active nodes in the hidden layer are found by the SA, the new nodes will be inserted to repair the prototype of the RBFNN. The parameters v, δ and w of the new nodes are given as follows (assuming the h-th node is the active node and only a new node will be inserted):

1. The position of the centers v; in order to speed up the convergence, the center of the new inserted node is given by the Nearest Neighbor Interpolation theory, and the position of the center is: $v = \frac{1}{2} v_h + v_{nearest}$. $v_{nearest}$ is the nearest node of the active node.
2. The width δ of the centers; finding out the minimal value of δ within the existing nodes, and the width of the new node is: $\delta = \min(\delta_i)$.
3. The weights w; in order to speed up the convergence and keep the least error of the next output of the RBF. The weights w of the new node is: $w = 0.5 \times w_{act}$, w_{act} is the weights of the active node.

By adding a new node to the hidden layer, the number of the nodes in hidden layer will be updated, the centers, width and the weights should be updated for the whole RBFNN. Since the new node is inserted based on the activity of the node for the output, the new node can ensure the activity for the whole network.

①For each factor Z_h, finding its minimal and maximal values a_h and b_h.
②Setting the interference factor to M and choose the number of simulation runs N.
③Computing the frequency $\omega_h = (N-1)/2M$ to be assigned to the factor Z_h.
④Just considering the output of the hidden nodes in the RBFNN, the factors are varied according to the curve definition; computing the total sensitivity index ST_h of the factor Z_h based on formula (20).
⑤Computing the percentage contribution $ST_h \Big/ \sum_{i=1}^{p} ST_i$ of the output of each hidden node.
⑥If the percentage contribution $ST_h \Big/ \sum_{i=1}^{p} ST_i$ larger than ε_1, the node h is the active node and a new node should be inserted with relation to the active node.
⑦Repeating ①-⑥ until all of the existing hidden nodes are considered.

Table 1. Procedure of selecting hidden nodes.

c. Parameters adjusting

After adding new nodes to the RBFNN, the number of the centers is confirmed timely. Then the whole parameters of the RBFNN will be adjusted. In fact, these parameters adjusting relate to the final capabilities of the RBFNN directly.

Considering the training process of the RBF, researchers have put forward many methods to adjust the parameters of the RBFNN. The parameter adjusting algorithm is based on the mean squared error (MSE) in this chapter:

$$E(t) = \frac{1}{T} \sum_{t=1}^{T} (y_d(t) - y(t))^2 \tag{21}$$

Where, T is the total number of the samples, $y_d(t)$ is the expected output of the t step, $y(t)$ is the practical output of the t step. The goal of this method is to reach $E(t) < \varepsilon$ by learning. ε is the expected stable error. The details of the adjusting process are:

4. The weights w ;

$$w_{ij}(t+1) = w_{ij}(t) - \eta_1 \frac{\partial E(t)}{\partial w_{ij}(t)} \tag{22}$$

$i = 1,2,\cdots,p$; $j = 1,2,\cdots,m$.

η_1 is a plus constant, and it is less than 1.

5. The width δ of the centers;

$$\delta_i(t+1) = \delta_i(t) - \eta_2 \frac{\partial E(t)}{\partial \delta_i(t)} \quad i = 1,2,\cdots,N ; \tag{23}$$

η_2 is a plus constant, and it is less than 1.

6. The position v of the centers;

In section B, the position v of the new inserting nodes has been discussed, and the position v of the other centers will be discussed here.

$$v_i(t+1) = \begin{cases} v_i(t) - \eta_3(P(k) - v_i(t)) & \text{if } v_i \text{ is active} \\ v_i(t) - \eta_4 \dfrac{\partial E(t)}{\partial v_i(t)} & \text{others} \end{cases} \quad i = 1,2,\cdots,p \tag{24}$$

η_3, η_4 are the plus constants, which are less than 1; p is the whole number of the hidden nodes after adding new nodes. This new algorithm will continue to circulate until reach the stable error.

d. Repair RBFNN

Choose the parameters M , N , ε_1 , η_1 , η_2 , η_3, η_4 ; initialize the centers v , The width δ , The weights w and the number of the hidden nodes p . In each sampling period, the main steps of the repair RBFNN algorithm are shown as table 2:

Training a given RBF for some epochs.
Finding out the active nodes in the hidden layer and go to step ; if there is no active node, go to step.
Adding new nodes to the RBFNN.
Adjusting the parameters of the RBFNN and updating the whole value of the parameters.
Repeating the step - , Stopping computing until the RRBF achieves the expected stable MSE.

Table 2. Procedure of RRBF neural network

In this proposed repair RBF algorithm, two points need to be highlighted. First, usually, the former dynamic RBFNN used the clustering methods or based on the information matrix, these methods required heavy computation. The SA method used in this chapter is based on the Fourier translation, and then the percentage contribution of the hidden neurons is computed in a quantitative way. This repair does not have to judge for the structure every step, therefore gives a completely satisfactory method for growing the hidden nodes. Second, the common SA method is based on the quantitative and qualitative methods and between local and global techniques. The SA method used in this chapter is global, and the percentage contribution of the hidden neurons is direct related to the RBF output.

3.3 Simulations

To demonstrate the effectiveness of the proposed algorithm, three examples are discussed in this chapter: nonlinear function approximation, dynamic system identification. The results are compared with other algorithms such as SGP-RBF (M. J. Er & S. Wu, 2002), and GAP-RBF (P. Hao & J. Chiang, 2006).

a. Tracking Nonlinear Function

Consider a common nonlinear function which was also used in (Gang Lengl et al., 2004) to demonstrate the effect of the algorithms:

$$y = 1.1 \times (1 - x_1 + 2{x_2}^2) \times e^{(-{x_3}^2/2)} \tag{25}$$

where there are 3 continuous attributes x_i ($i = 1,2,3$). And the data set $\{x_i; y\}$ is generated by the equation (25), and x_i satisfies the uniform distribution U [0, 10]. For each trial, the size of training samples is 200, and the size of testing samples is 200.

The real output at time k is $y(k)$, the required value at time k is $y_d(k)$, the error at time k will be $e(k) = y_d(k) - y(k)$. The inputs of this SORBF are given as: $P(k) = (x_1(k), x_2(k), x_3(k))$. The training MSE for tracking is 0.001, the initial radius of every hidden node is 0.1; the initial weight of every hidden node is randomly given in the interval [0, 1]. The initial value of M and N are $M = 4$ and $N = 5000$. There are two initial nodes in the hidden layer.

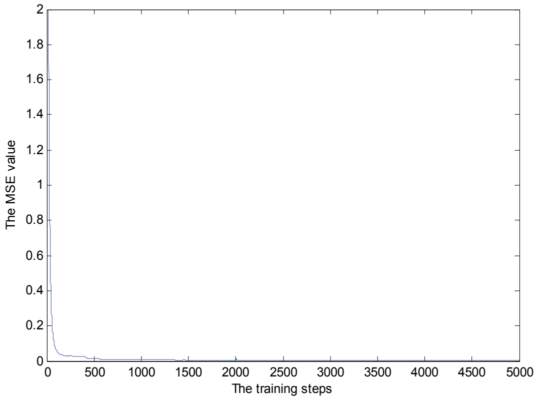

Fig. 2. The error results in the tracking process

Fig 2 shows 5000 steps of the error values in the training process, the error values show that when the new node is inserted to the hidden layer, the error values will shake. However, the error values can be convergence quick after adding new nodes; Fig 3 shows the dynamic number of the nodes in the tracking process; Fig 4 shows the width δ of the centers after training; Fig 5 shows the RBF position of the centers v after training.

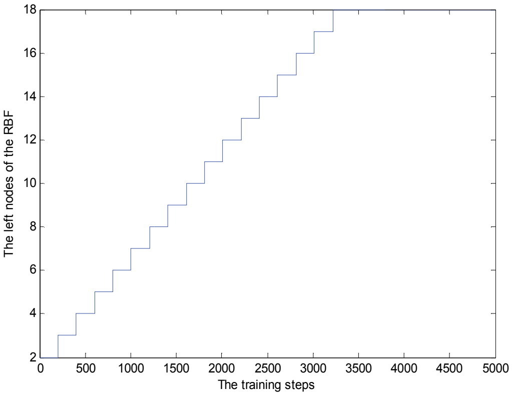

Fig. 3. The number of the nodes in the tracking process

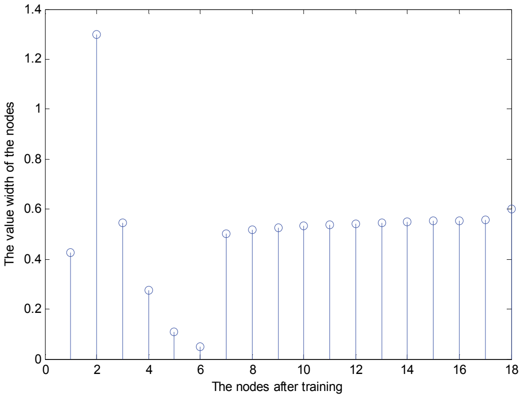

Fig. 4. The δ values of the left nodes after training

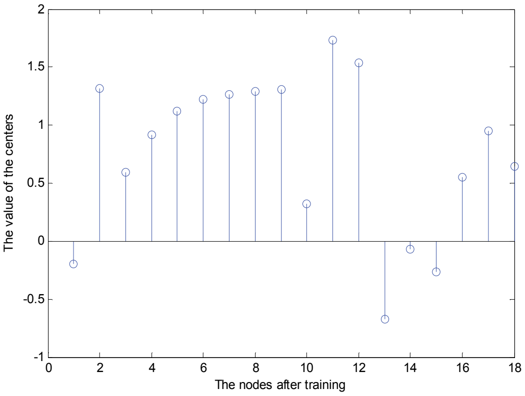

Fig. 5. The v values of the left nodes after training

The final results of all the algorithms are shown in Table 3. The compared values are: training time, test error, left nodes after training.

Algorithm	CPU Time(s)	Training Error	Testing Error	No. of Neurons
SGP-RBF	42.32	0.001	0.0086	19
GAP-RBF	26.86	0.001	0.0031	16
RRBF	22.67	0.001	0.0025	18

Table 3. The performances comparison of the different algorithms

Based on table 3, the RRBF is faster and more accurate than SGP-RBF and GAP-RBF. The structure of this RRBF is simpler than the SGP-RBF; the memory space is fewer owing to the simple structure. The nodes in the RRBF are more than the GAP-RBF, but the algorithm is faster and more accurate than GAP-RBF. The results prove that this RRBF performs better than the former two algorithms and it has better ability to model the nonliear systems.

4. Experimental results of COD soft-sensing

The widespread popularity of neural networks in many fields is mainly due to their ability to approximate complex nonlinear mappings directly from the input samples. They can solve many problems especially in modelling which are difficult to handle using classical parametric techniques. Since the activated sludge wastewater treatment process is a high nonlinear and complicated system, it is very suitable to be modeled by neural network. In the experiment, we select the most important influent water quality parameters COD, Mixed Liquor Suspended Solid (MLSS), pH, Oil and NH3-N as the research variables, where COD measures the total amount of oxygen that can be combined with the chemical compounds (organic and inorganic) in the water. For it is an extremely important value to reveal the total amount of pollution in water and is easily to acquire, it is widely used in the wastewater treatment process. MLSS is a measure of dry solids concentration in mg/l in mixed liquor in an aeration tank. PH shows the degree of acid or alkali in the influent water, Oil is the content of oil contamination, and NH3-N delegates the content of nutritious contamination in the influent water. The output vector of the network is the value of COD of the water flow out of the system after the treatment. By the way, the data used in the experiment are from a small wastewater treatment factory.

The model used the data of SS, pH, oil and NH3-N as inputs to estimate the settled sewage COD. Thus, the data set used to develop the model consisted of 100 samples of the model inputs and settled sewage COD and 100 samples for testing. Hence, the model has been developed to determine if acceptable estimates can be produced from a limited amount of relatively inexpensive and readily available information. Note also that for each set of input data the model is estimating the corresponding COD. It is used on-line then each model would give an instantaneous estimation of the COD that could be expected if standard laboratory tests were performed. Each model input was scaled to lie in the range 0 to 1 to reduce the effects of widely differing magnitudes of input data that could lead to a biased model. The model output was scaled in an identical fashion. The error measures for this model are 3mg/L confidence limits. The results are shown Figs. 6–11.

Fig.6 gives the training results of COD; Fig.7 describes the error value of the trained results which are less than 3mg/L; Fig.8 shows the left nodes in the hidden layer after training; Fig.9 describes the error value in the training process; Fig.10 shows the predictions results of COD; Fig.11 shows the error value of the predicting process.

Based on the results, this RRBF is able to be used for the COD measurement on-line. The results demonstrate that the COD trends in the settled sewage at the wastewater treatment could be predicted with acceptable accuracy using SS, pH, Oil and NH_3-N data as model inputs. This approach is relatively straightforward to implement on-line, and could offer real-time predictions of COD. It is concluded that this is a significant feature of this approach since COD is the more commonly used and readily understood measure.

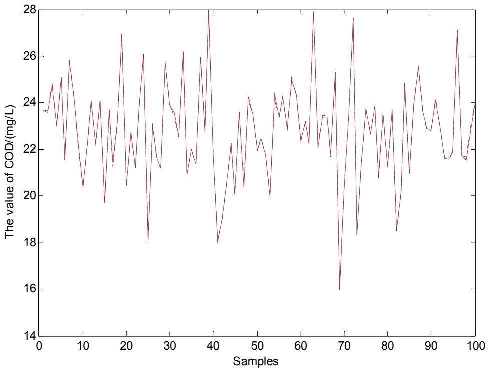

Fig. 6. The training results of COD

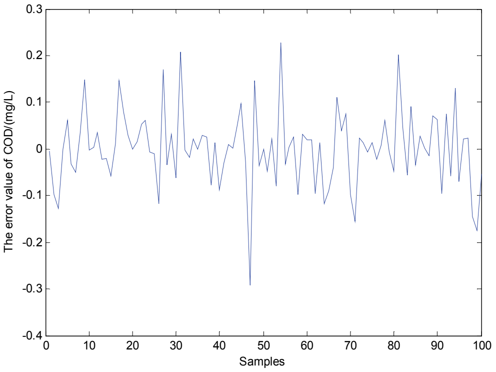

Fig. 7. The error value of the trained results

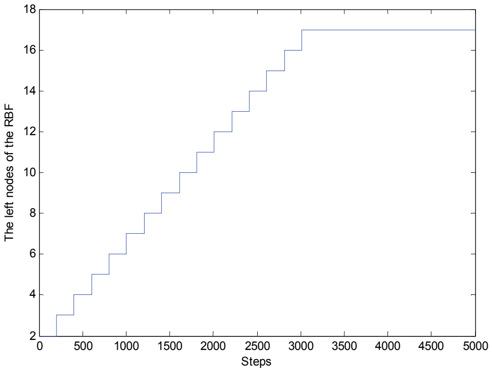

Fig. 8. The number of the nodes in the training process

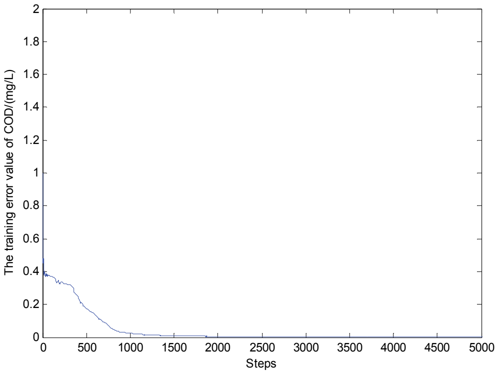

Fig. 9. The error value of the training process

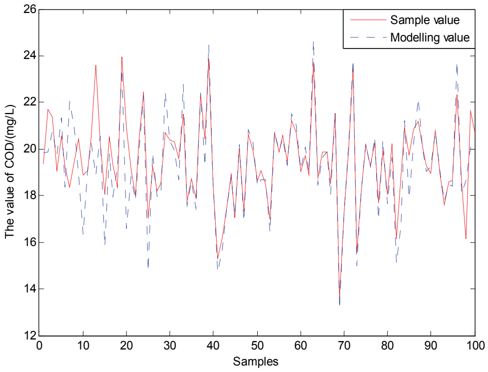

Fig. 10. The predictions results of COD

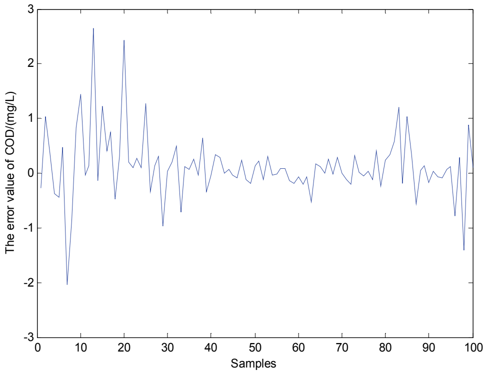

Fig. 11. The error value of the predictions results

5. Conclusion and future work

Section 3 presents a repair algorithm for the design of a RBF neural network which is called RRBF to model the COD in wastewater treatment process.

The following important points should be noted:

1. In most algorithms the criterion used to determine growth is dependent on the current time (t + m). This section, however, uses the sensitivity index, which can calculate the contributions of hidden nodes over a number of time periods (t + 1, t + 2, . . . , t + m). This is more objective than using a criterion based on the current time (t + m).
2. The criterion used to select hidden nodes is based on the SA method of the RBF output – it is independent of the input data.
3. Less computation is required because the initial weights of the new inserted nodes are utilized to calculate the repaired RBF. Simulation results show that the proposed algorithm performs well in modelling the key parameter, COD, in the wastewater treatment process. This type of RRBF based approach may potentially be used in any area where it is difficult to measure a range of variables because of the need for specialized equipment. It can, therefore, be a cost effective solution in many application areas where such measurements are needed.

The following future work is under investigation.

1. An adaptive repairing strategy which will allow the addition of hidden nodes during the training process based on the SA of the network output.
2. A pruning operation which will reduce the hidden nodes that have little contribution to the output of the RBF network is under investigation.
3. The application of the algorithm to other areas is also on-going.
4. The growing Mechanism need further improvement.

6. Reference

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6

Formaldehyde Oxidizing Enzymes and Genetically Modified Yeast *Hansenula polymorpha* Cells in Monitoring and Removal of Formaldehyde

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1. Introduction

Formaldehyde (FA), a very important commercial chemical, is one of the most toxic pollutants used in many industries. It is exploited as an adhesive material in pressed wood products, as a preservative in paints and coatings, in the production of fertilizers, paper and plywood, urea-formaldehyde resins and numerous other applications (Yocom Y.E., 1991; Otson, 1992; Khoder, 2000). It is also applied in the production of cosmetics and sugar, in well-drilling fluids, in agriculture as a preservative for grains and seed dressings, in the rubber industry in the production of latex, in leather tanning and in photographic film production. FA has been a popular constituent of embalming solutions since about 1900 (Kitchens et al., 1976; Plunkett and Barbella, 1977). Approximately 30 years following its discovery, FA was introduced into medical practice as a disinfectant and tissue hardener, used in many hospitals and laboratories to preserve tissue specimens (Walker, 1964; Cox, 1984). It has medical applications as a sterilizer and is employed as an anti-viral agent and preservative in the production of vaccines, instead of the harmful merthiolate, which can cause neurodevelopmental disorders including autism and autism spectrum disorders (Offit, 2007; Geier, 2004).

FA has a negative influence on human health, especially on the central nervous, blood and immune systems. Anatomists, technicians, medical or veterinary students and embalmers are among the people who have a great risk for FA toxicity. FA can also be found in the air that we breathe at home and at work, in the food we eat, and in some products that we put on our skin. A major source of FA that we breathe everyday is found in smog in the lower atmosphere. Automobile exhaust from cars without catalytic converters or those using oxygenated gasoline also contain FA (Kitchens et al., 1976; National Research Council, 1982). At home, FA is produced by cigarettes and other tobacco products, gas cookers, and open fireplaces. It is found in many products used every day around the house, such as antiseptics, cosmetics, dish-washing liquids, fabric softeners, shoe-care agents, carpet cleaners, glues, lacquers, paper, plastics, and some types of wood products (Gerberich and Seaman, 1994). Inhaled FA primarily affects the airways; the severity and extent of the physiological response depends on its concentration in the air. Acute inhalation exposure to FA causes histopathologic damage (Chang et al.,1983) and DNA-protein cross-linking in the nasal mucosa of rats and rhesus monkeys (Auerbach et al.,1977; Martin et al.,1978; Griesemer et al.,1982; Casanova et al.,1989). Recently, a new health risk factor associated with FA has been revealed. Some advanced technologies of potable water pre-treatment include the ozonation process, during which FA is generated as a result of the reaction of ozone with humus traces (Schechter and Singer, 1995). FA has been in widespread use for over a century as a preservative agent in some foods, such as some types of Italian cheeses and dried foods. It has been found as a natural chemical in fruits and vegetables, and in human flesh and biological fluids (Gerberich and Seaman, 1994). In extreme cases, some frozen fish, especially of the *Gadoid* species, can accumulate up to 200 mg of FA per kg of wet weight due to the enzymatic degradation of a natural fish component - trimethylamine oxide (Rehbein, 1995; Pavlishko et al., 2003).

FA is classified as a mutagen and possible human carcinogen (Feron et al., 1991), one of the chemical mediators of apoptosis. FA is clearly genotoxic *in vitro*. It induces mutations and DNA damage in bacteria. DNA-protein cross-links, DNA single-strand breaks, chromosomal aberrations, sister chromatid exchanges and gene mutations are induced in human and rodent cells. Animal studies indicate that FA is a rat carcinogen at high levels ($\geq$ 10 ppm) of exposure, producing nasal tumours that are both exposure duration and concentration-dependent (Shaham J. et al., 1996.

At the same time, FA is a naturally occurring metabolite produced in very small amounts in our bodies as part of our normal, everyday metabolism of serine, glycine, methionine and choline and also by the demethylation of *N*-, *S*- and *O*-methyl compounds (Heck, 1984). It is estimated that endogenous FA concentration in blood is close to 0.1 mM. FA may be detoxified principally via action of formaldehyde dehydrogenase (FdDH, EC 1.2.1.1), a specific enzyme that catalyzes the conversion of FA in the presence of reduced glutathione (GSH) and NAD^+ to S-formylglutathione (finally, to formic acid) and NADH (Uotila and Mannervik, 1979; Pourmotabbed and Creighton,1986). S-formylglutathione (GSCH=O) is finally hydrolyzed to free formic acid:

$$CH_2O + GSH \leftrightarrow GS\text{-}CH_2OH \qquad (1)$$

$$GS\text{-}CH_2OH + NAD^+ \xleftrightarrow{FdDH} GS\text{-}CH{=}O + NADH + H^+ \qquad (2)$$

$$H_2O + GS\text{-}CH{=}O \longrightarrow GSH + HCOOH \qquad (3)$$

Since FdDH is a glutathione dependent enzyme, the pool of glutathione available for FA binding is important in regulating FdDH activity. Then FA can be metabolised to formate and enter the one carbon pool for incorporation into the cells constituents (Casanova-Schmitz, 1984). At the moment, three different FdDHes, bacterial NAD$^+$-dependent, yeast NAD$^+$- and GSH-dependent and bacterial dye-linked NAD$^+$ and GSH-independent, are widely used for bioanalytical purposes (Ben Ali et al., 2006, 2007; Winter and Cammann, 1989; Vastarella and Nicastri, 2005; Herschkovitz et al., 2000; Korpan et al., 1993; Gonchar et al., 2002; Korpan et al., 2010; Achmann et al., 2008; Kawamura et al., 2005).

Besides FdDH, FA can be easily oxidized by alcohol oxidase (AOX) (EC 1.1.3.13), an enzyme which is responsible *in vivo* for the first reaction of methanol metabolism in methylotrophic yeast (Klei van der et al, 1990). AOX is not an absolutely selective enzyme and oxidizes the hydrated form of FA to formic acid without any exogenous cofactor (Kato et a*l*., 1976). The theoretical possibility of AOX using for FA assay is based on a known fact that FA exists in aqueous solutions in the hydrated form (95–99% of total concentration) which has a structural resemblance to methanol and can be oxidized by AOX` with the subsequent formation of formic acid and hydrogen peroxide according to the following reactions:

$$CH_2O + H_2O \leftrightarrow HOCH_2OH \tag{4}$$

$$HOCH_2OH + O_2 \xrightarrow{AOX} HCOOH + H_2O_2 \tag{5}$$

2. Methods of formaldehyde monitoring

2.1 Chemical and enzymatic methods

There are many chemical methods for the determination of FA (Sibirnyi et al., 2005; Bakar et al., 2009). The traditional Nash's method (Nash, 1953) is based on the reaction of FA with acetylacetone in the presence of ammonium ions. Another widely used photometric and sufficiently sensitive analytical method exploits the reaction of FA with chromotropic acid (Sawicki et al., 1962). This approach enables the determination of the analyte in the concentration range 0.05 - 1.0 mg dm^{-3} (Polish Standard, 1988). Unfortunately, determination of FA involves heating the sample with chromotropic acid under strongly acidic conditions.

4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (AHMT) was also proposed for FA assay (Avigad, 1983, Jung et al., 2001). FA and other aldehydes form products of different colors, which can be selectively tested spectrophotometrically. The sensitivity limit of the method is 1.5 nmol of FA in 1 ml sample. However, the main drawback of the AHMT method is the requirement of a very strong base.

High Performance Liquid Chromatography coupled to steam distillation and 2,4-dinitrophenylhydrazine derivatization (2,4-DNPH) displayed good selectivity, precision and accuracy (Li et al., 2007).

A polarographic method has been developed for the determination of FA traces by direct *in situ* analyte derivatization with (carboxymethyl)trimethyl ammonium chloride hydrazide (Girard T-reagent) (Chan & Xie, 1997). The drawback of this method is the expensive apparatus required, as well as the necessity to remove oxygen traces by sparging with pure nitrogen.

A flow injection analysis (FIA) system with an incorporated gel-filtration chromatography column has been applied to determine FA using FdDH (Benchman, 1996).

2.2 Biosensor methods

The degree of selectivity or specificity of a given biosensor is determined by the type of biocomponent incorporated into the biosensor. Biological recognizers are divided into 3 groups: biocatalytic, bioaffinity and hybrid receptors (Mello and Kubota, 2002). The selection of an appropriate immobilization method depends on the nature of the biological element, type of transducer used, physico-chemical properties of the analyte and operating conditions of the biosensor system (Luong et al., 1988). Biosensors can be categorized according to their transducer: potentiometric (Ion-Selective Electrodes (ISEs), Ion-Sensitive Field Effect Transistors (ISFETs)), amperometric, conductometric, impediometric, calorimetric, optical and piezoelectric.

FA selective biosensors are based on cells (Korpan et al., 1993) or enzymes used as biorecognition elements: either alcohol oxidase (AOX) (Korpan et al., 1997, 2000; Dzyadevych et al., 2001) or formaldehyde dehydrogenase (FdDH) (Herschkovitz et al., 2000; Kataky et al., 2002, Achmann et al., 2008). A number of sensor approaches for the detection of FA concentration have been published including systems operating in gas (Dennison et al., 1996; Hämmerle et al., 1996; Vianello et al., 1996) and organic phases. An optical biosensor has also been proposed for FA assay (Rindt & Scholtissek, 1989).

Potentiometric biosensors, consisting of a pH sensitive field effect transistor as a transducer and either the enzyme AOX, or permeabilised yeast cells (containing AOX) as the biorecognition element, have been described by Korpan et al. (2000). These biosensors have demonstrated a high selectivity to FA with no interference response to methanol, ethanol, glucose or glycerol.

Amperometric biosensors have been suggested for the determination of FA level using FdDH (Winter & Cammann, 1989; Hall et al., 1998). Conductometric enzymatic biosensors based on FdDH (Vianello et al., 2007) and AOX (Dzyadevych et al., 2001) have been developed for FA assay.

3. Microbial methanol and formaldehyde biodegradation in wastewater

The study of microbial methanol and FA biodegradation in wastewater is an important problem of environmental biotechnology. Different microorganisms are capable of FA degradation: bacteria *Pseudomonas* spp. (Kato et al., 1983), *Halomonas spp*. (Azachi et al., 1995) and various strains of *Methylotropha* (Attwood & Quayle, 1984); the yeasts of genera *Debariomyces* and *Trichosporon* (Kato et al., 1982), *Hansenula* (van Dijken et al., 1975), *Candida* (Pilat & Prokop, 1976) and the fungi *Gliocladium* (Sakagushi et al., 1975). Selected strains of *Pseudomonas putida*, *Pseudomonas cepacia*, *Trichosporon penicillatum* and the mixed culture of these three species were used for aerobic degradation of FA and formic acid in synthetic medium and wastewater generated by melamine resin production (Glancer-Šoljan et al., 2001). The selected mixed culture containing two bacterial strains of *Pseudomonas* (*P. putida and P. cepacia*) and *Trichosporon* yeast genera (*T. peicillatum*) exhibited highly efficient degradation of FA and formic acid in the synthetic medium. The mixed culture also degraded formaldehyde, methanol and butanol contained in the wastewater of the melamine resin production facility.

Nineteen bacterial strains able to degrade and metabolize FA as a sole carbon source were isolated from soil and wastewater of a FA production factory. The samples were cultured in complex and mineral salts media containing 370 mg FA/L. Some strains were identified to be *Pseudomonas pseudoalcaligenes*, *P. aeruginosa*, *P. testosteroni*, *P. putida*, and *Methylobacterium extorquens*. After adaptation to high concentrations of FA, microorganisms completely consumed 3.7 g FA/L after 24 h, and degraded 70% of 5.92 g FA/L after 72 h (Mirdamadi et al., 2005). The development of appropriate technologies for the treatment of FA discharged into the environment is important to minimize its negative impact. Studies have shown that in a special reactor for treating FA, both *Methanosaeta* and *Methanosarcina* were found to thrive with influent FA concentrations higher than 394.0 mg HCHO/L. Microorganisms like *Methanosaeta* probably survived due to its preferential use of acetate while *Methanosarcina* preferentially used the methanol (Oliveira et al., 2004). Biodegradation of FA was also tested in the marine microalga *Nannochloropsis oculata* (Yoshida et al., 2009). Transformation of [^{13}C]-FA in the medium was monitored by nuclear magnetic resonance (NMR) spectrometry. FA was transformed into formate, and these two substances degraded in the medium as was clearly shown by the NMR spectrometry.

Environmental FA can be detected and remediated in a biological system that incorporates a bacterium *Rhodobacter sphaeroides* containing suitable genetic sequences encoding a FA-inducible regulatory system. The system includes a transcriptional promoter from *Rhodobacter sphaeroides* that can be specifically induced in the presence of FA to transcribe an operable linked gene (US Patent 6242244).

The application of the methylotrophic yeast *Hansenula polymorpha* to the treatment of methanol and FA containing wastewater was experimentally verified. A variety of wastewater samples originating from chemical industry effluent were examined (Kaszycki & Kołoczek, 2000; Kaszycki et al., 2001). The methylotrophic yeast *H. polymorpha* was shown to cooperate with activated sludge from biological wastewater treatment stations, enhancing substantially its potential to biodegrade FA in industrial wastewater. After integration with yeast cells, the modified sludge retained its original structure and activity whereas its resistance to elevated FA concentrations was significantly improved (Kaszycki & Koloczek, 2002). An yeast isolate revealing unique enzymatic activities and substrate-dependent polymorphism was obtained from the autochthonous microflora of soil heavily polluted with oily slurries. By means of standard yeast identification procedures, the strain was identified as *Trichosporon cutaneum*. Further molecular PCR product analysis of ribosomal DNA confirmed the identity of the isolate with the genus *Trichosporon*. As it grew on methanol as a sole carbon source, the strain appeared to be methylotrophic, able to utilize formaldehyde (Kaszycki et al., 2006).

Mitsui et al. (2005) isolated a bacterial strain that efficiently degraded FA and used it as a sole carbon source. The isolated strain was identified as *Methylobacterium* sp. MF1, which could grow on FA and methanol. The resistance to the toxic effects of FA exhibited by *Methylobacterium* sp. MF1 is related to factors other than C1 metabolism.

Microorganisms utilizing methanol have adopted several metabolic strategies to cope with the toxicity of FA. Mechanisms of FA detoxification in yeast, bacteria and archaea were studied (Yurimoto et al., 2005). The toxicity of FA in batch assays, using volatile fatty acids as co-substrates and the continuous anaerobic treatment of wastewaters containing FA in upflow anaerobic sludge blanket reactors was investigated (Vidal et al., 1999). The kinetic process of FA biodegradation in a biofilter packed with a mixture of compost, vermiculite powder and ceramic particles was studied by Xu et al. (2010).

4. FA-oxidizing yeast enzymes for FA monitoring

4.1 NAD$^+$- and glutathione-dependent formaldehyde dehydrogenase (FdDH)

4.1.1Yeast engineered for overproduction of FdDH

To construct strains of *H. polymorpha* that over-produce thermostable NAD$^+$- and glutathione-dependent FdDH, the *H. polymorpha FLD*1 gene with its own promoter

(Baerends et al., 2002) was inserted into the integrative plasmid pYT1 (Demkiv et al., 2005) containing the *LEU2* gene of *Saccharomyces cerevisiae* (as a selective marker). The constructed vector was used for multi-copy integration of the target gene into the genome of *H. polymorpha* by transformation of *leu 1-1* (Demkiv et al., 2005) and *leu 2-2* recipient cells (both *leu* alleles are complemented by *S. cerevisiae* gene *LEU2*). The transformation was performed using three different methods (Table 1): electroporation (Delorme, 1989), the lithium chloride method (Ito et al., 1983), and the protoplasting procedure (Hinnen et al., 1978).

Selection of FdDH-overproducing strains was carried out simultaneously by leucine prototrophy and by resistance to elevated FA concentrations in the medium. Of more than 150 integrative Leu$^+$- transformants with higher resistance to FA – up to 10-12 mM on solid plates, 14 stable clones, resistant up to 15-20 mM FA on plates, were selected and studied in more detail. The growth characterstics of selected clones in the liquid medium were shown in Fig.1: all transfomants grew better and were more resistant to elevated FA content in liquid medium with 1% methanol, compared to the recipient strains (Demkiv et al., 2005, Gayda et al, 2008). Finally, FdDH specific activities were tested in cell-free extracts (CE) of the best selected FA-resistant Leu-prototrophic transformants (Fig. 2).

Parental strains	Transformation method	Plasmid	Number of experiments	Average transformation efficacy, Leu$^+$-clones/µg DNA	Number of the tested clones with a higher resistance to FA
Leu1-1	Electroporation	pHpFLD1	3	2x10^3	12
Leu2-2		pHpFLD1	3	30	10
Leu1-1		pHp(FLD1)$_2$	3	1.5x10^3	50
Leu2-2		pHp(FLD1)$_2$	3	15	10
Leu1-1	LiCl	pHpFLD1	3	2	12
Leu2-2		pHpFLD1	3	20	80
Leu1-1	Protoplastes	pHp(FLD1)$_2$	1	0.5	1
Leu2-2		pHp(FLD1)$_2$	1	0.4	2

Table 1. Efficacy of different transformation methods for two strains of the yeast *H. polymorpha* by plasmids *pHpFLD1* and *pHp(FLD1)*$_2$

Activity of FdDH was determined by the rate of NADH formation monitored spectrophotometrically at 340 nm (Schutte et al., 1976). One unit (1 U) of the enzyme activity was defined as the amount of the enzyme which forms 1 µmole NADH per min under standard conditions of the assay: 25^{o}C, 1 mM FA, 1 mM NAD$^+$, 2 mM GSH in 50 mM Phosphate buffer (PB, pH 8.0).

Tf 11-6 and Tf-142 were the most effective recombinant strains, with the highest FdDH activity, up to 4.0 U/mg, which is a 4-5 fold increased as compared to the parental strains, *leu* 1-1 and *leu* 2-2, respectively. These transformants were characterized and chosen as a source for FdDH production. It was estimated by Southern dot-blot analysis, that genomes of the stable recombinant yeast clones contain 6-8 copies of the target *FLD1* gene, which confirmed the results obtained by the Southern-hybridization method (data not shown). Therefore, the recombinant yeast strain Tf 11-6 contains more than 8 copies of the integrated plasmid, as compared to 1 copy of the parental strain, probably due to the usage of the double-gene plasmid *pHp(FLD1)*$_2$ and its tandem integration into the genome of the recipient strain.

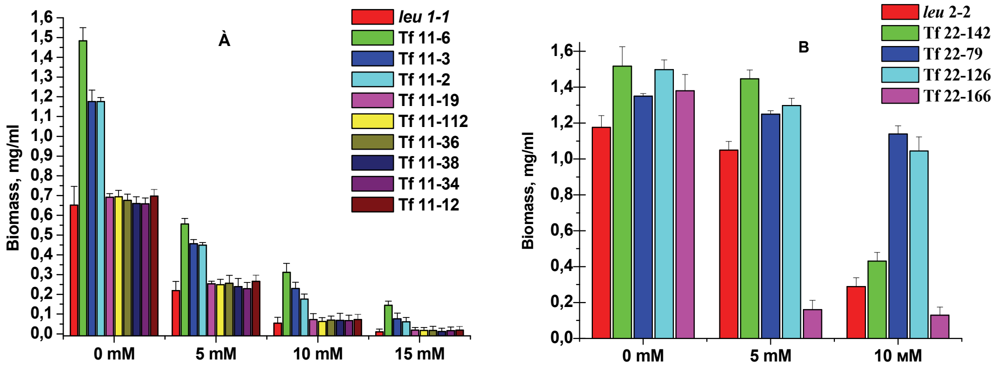


Fig. 1. Resistance to FA of the recipient yeast strains *leu1-1*(A) and *leu2-2* (B), *of H. polymorpha* and their transformants, grown in 1% methanol medium

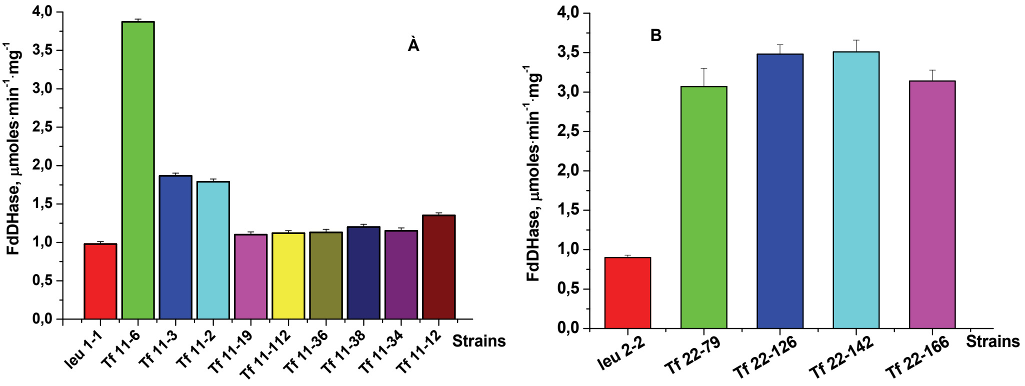


Fig. 2. Specific activity of FdDH in cell-free extracts of parental yeast strains *leu1-1*(A) and *leu 2-2* (B) *of H. polymorpha* and their transformants grown in 1% methanol medium

4.1.2 Optimization of cultivation conditions for FdDH-overproduction

In order to optimize cultivation conditions to obtain the highest enzyme yield, the influence of growth medium composition on FdDH concentration using the two best strains, Tf 11-6 and Tf 22-142, was studied. FdDH activity in cell-free extract was dependent on a carbon source. Cultivation in 1% methanol as a sole carbon source resulted in the highest levels of the enzyme synthesis for both of the tested strains (Fig. 3), which is in accordance with the literature concerning the wild type strains (Hartner et al., 2006; Harder et al.,1989; Egli et al.,1982).

The addition of FA to the methanol medium stimulated synthesis of FdDH. Under experimentally determined optimal conditions, *i.e.* methanol as carbon source, methylamine as nitrogen source and 5 mM FA as an additional inductor of FdDH synthesis, target

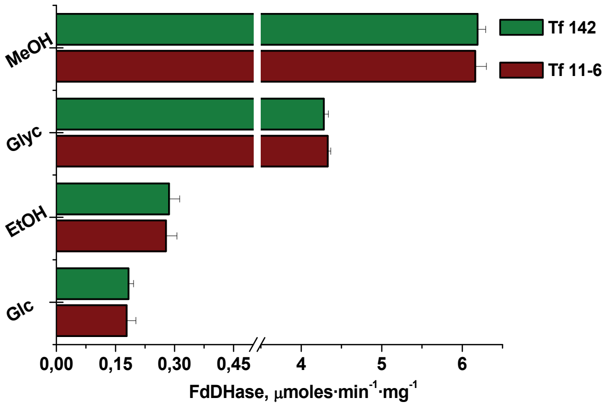


Fig. 3. FdDH activity in CE of the recombinant strains Tf 11-6 and Tf 142, cultivated on the media with methylamine, 5 mM FA and different carbon sources: 1% ethanol (EtOH), 1% methanol (MeOH), 1% glucose (Glc) or glycerol (Glyc).

enzyme activity achieved was 6.2 U/mg, 1.6-fold higher than under normal growth conditions, as described in Fig. 2. The addition of up to 10 mM FA to the optimal culture medium resulted in FdDH activity of 8.3 U mg^{-1}, a 2-fold increase as compared to medium without FA (Fig.2). The strong correlation between FA concentration in the medium and FdDH activity in cultivated cells of recombinant yeast strain Tf-11-6, demonstrates the important role of FA as a FdDH-synthesis inducer (Fig. 4).

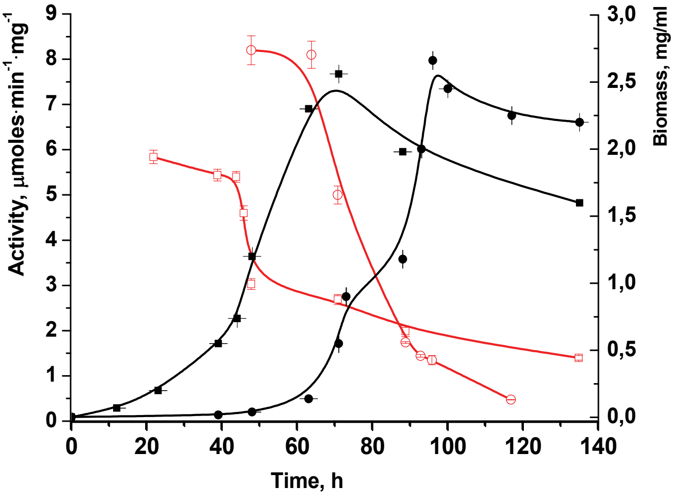


Fig. 4. FdDH activity (red), and biomass (black) of the enzyme-overproducer Tf-11-6 during cultivation in a medium with 1% methanol supplemented with 5 mM (■, □) and 10 mM (●, ○) formaldehyde.

4.1.3 FdDH purification and characterization

For enzyme isolation from cell-free extracts, cells of the recombinant over-producer strain Tf 11-6, cultivated in 1 % methanol medium supplemented with 5 mM FA during 20 h, were used. A simple scheme for FdDH isolation and purification on anion-exchange sorbent was proposed, resulting in a FdDH preparation with specific activity about 27 U units per mg of protein. For comparison, specific activities of commercially available FdDH preparations from *Ps. putida* and from the yeast *C. boidinii* are 3-5 U mg^{-1} and 17-20 Umg^{-1}, respectively (Demkiv, et. al. 2007). The purity of the isolated enzyme preparation was controlled by PAAG electrophoresis in denaturizing conditions (Laemmly, 1970).

Some physico-chemical characteristics of the purified FdDH are shown in Table 2.

Strains/ property		***Candida boidinii***	***Pichia pastoris***	***H. polymorpha*** strains	
				wild type	recombinant Tf11-6
M, kDa	Enzyme	80/82	84	82	-
	Subunit	40 /42	39/41	40.6	40
K_M, mM	FA	0.25/0.29	0.43/0.31	0.21	0.18
	GSH	0.13/-	0.48/0.16	0.18	-
	NAD$^+$	0.09/0.025	0.24/0.12	0.15	0.21
	Methylglyoxal	1.2/2.8	-	-	-
	Formylglutation	-/0.12	-/0.6	-	-
	NADH	-/0.025	-/0.25	-	-
Temperature optimum, ^{0}C		35/-	47/-	-	50
Thermostability, ^{0}C*		52/-	52/-	-	57
pH optimum		8.5/-	7.9/-	8.2	7.5-8.5
Reference		Schutte et al., 1976 /Kato et al., 1990	Allais et al.,1983; /Patel et al., 1983	Uotila et al., 1979	Demkiv et al., 2007

Table 2. Comparison of structural and enzymatic properties of FdDH.

The molecular mass of the FdDH subunit, estimated by SDS-electrophoresis, was shown to be approximately 40 kDa, similar to the 41 kDa found for *C. boidinii* (Melissis et al., 2001). It was reported that the predicted *FLD1* gene product (Fld1p) is a protein of 380 amino acids (Baerends et al., 2002). Taking into account, that the M of the native enzyme from various methanol-utilizing yeasts were estimated to be from 80 to 85 kDa, isolated thermostable, NAD$^+$- and GSH-dependent FdDH can be assumed to be dimeric. As shown in Table 2, values of the Michaelis-Menten constant (K_M) for FA and NAD$^+$ calculated for this enzyme are close to K_M for the wild-type enzyme.

Optimal pH-value and pH-stability (during incubation in the appropriate buffer at room temperature for 60 min) of the enzyme were evaluated. Optimal pH was found to be in the range of 7.5-8.5, and the highest stability of FdDH was observed at pH 7.0-8.5.

The optimal temperature for enzyme activity was 50^{0}C. At 65^{o}C the enzyme retained about 60% of its highest activity (assay time 5 min), *i.e.* equal to the level of FdDH activity at 30^{0}C. The enzymatic activity at 37^{0}C was 1.6-fold higher than under the standard conditions of the FdDH activity assay (at 25^{0}C). Study of the thermal stability of the enzyme demonstrated that its activity was completely preserved after 10 min of incubation at 40^{0}C, and was partially preserved at 55^{0}C (up to 70%) and 60^{0}C (25%). Complete inactivation occurred after heating of the enzyme solution at 70^{0}C for 5 min. These results indicate that the thermostability of the enzyme is apparently high, enabling its usage for bioanalytical purposes, namely, for FA assay in food products, wastewater, and pharmaceuticals, as well as for biotransformation of FA to formic acid.

The effect of a number of inhibitors on the enzymatic properties was studied. Table 3 shows an influence of some compounds on enzymatic activity in purified FdDH preparation tested before and after its incubation with additives, for 30 min at 4°C. Bivalent cations ($Zn^{2+,}$ Cu^{2+} and Mn^{2+}), as well as an ionic detergent SDS were shown to inhibit FdDH activity. According to the literature, enzymes from two other yeasts, *P. pastoris* and *C. boidini* (Allais et al., 1983; Kato et al., 1990, Patel, 1983) were also inhibited in a similar fashion.

4.2 Enzymatic methods for FA monitoring

4.2.1 The development of FdDH- and AOX based enzymatic kits

FdDH preparation isolated from the recombinant strain of the yeast *H. polymorpha* with the specific activity 17.0 units per mg of protein at 25°C (that is about 27 U·mg^{-1} at 37°C) was proposed for the enzymatic assay of FA. In methylotrophic yeasts, NAD^+- and glutathione-dependent FdDH catalyzes the oxidation of FA to formic acid with the simultaneous reduction of NAD^+ to NADH.

Additive	FdDH activity (%) under different additives levels	
	1 mM	10 mM
$ZnSO_4$	23.3	0
$CuSO_4$	0	38.3
$FeCl_3$	78.3	0
$MnCl_2$	27.8	60.0
$MgCl_2$	84.8	85.0
EDTA	96.5	85.0
PMSF	91.7	56.3
2-mercaptoethanol	72.7	66.7
SDS	0	0
Dithiotreitol	96.33	85.2

Table 3. The influence of different additives, in concentrations 1 and 10 mM, on enzymatic activity of purified FdDH preparation (100 % activity has initial enzyme preparation)

The proposed enzymatic method is based on the photometric detection of colored product, formazan, formed from nitrotetrazolium blue (NTB) in reaction coupled to FdDH-catalyzed oxidation of FA in the presence of an artificial mediator, phenasine methosulfate (PMS) and detergent Triton X-100 (Demkiv et al., 2007, Demkiv et al., 2009):

$$NADH + NTB^{+} \longrightarrow NAD^{+} + \text{Formazan} \qquad (6)$$

The enzymatic kit "Formatest" was developed on the base of these reagents. The assay was performed in conditions of incomplete conversion of the analyte (approximately, 10 %), using a limited concentration of the enzyme (23 mU/ml) in the reaction mixture. These conditions are economic and reasonable, because of the high FA content in the tested samples. Under conditions of complete oxidation of FA (excess of the enzyme), assay sensitivity was determined to be 2.5 µM (in final reaction mixture) or 20 µM in the tested samples.

Alcohol oxidase (AOX) from the thermotolerant methylotrophic yeast cells *H. polymorpha* can be an alternative to FdDH, used for analytical purposes. This enzyme is quite stable, contains tightly bound FAD and does not need any exogenous co-enzyme for catalytic activity (Woodward J., 1990). Theoretically, AOX can be used to assay FA because in aqueous solutions FA exists in hydrated form (95–99% of total concentration) which structurally resembles methanol, and can be oxidized by AOX with the subsequent formation of formic acid and hydrogen peroxide (see reactions 4 and 5).

AOX preparations were isolated from the strain *H. polymorpha* C-105 - catalase-defective mutant (Gonchar et al., 1990) with impaired glucose catabolite repression of AOX synthesis (*gcr1, catX*). The mutant cells, grown in glucose medium, were disrupted and cell-free extract was used for partial purification of AOX by two-step ammonium sulfate precipitation (Gonchar et al., 1998). Using this simple procedure, enzyme preparation in a form of suspension in 60 % saturated $(NH_4)_2SO_4$, with specific activity of 7.5 U/mg, was obtained. This is close to activity of some commercial AOX preparations. As shown by PAAG electrophoresis, the isolated crude AOX preparation is not homogenous, but still suitable for analytical application. AOX preparation can be stored at 4 °C in 60 % saturated ammonium sulfate in the presence of protease inhibitors for at least 1 year without loss of activity.

The oxidase–peroxidase-based method (AOP) and enzymatic kit "Alcotest" were developed on the base of two enzymes - alcohol oxidase (AOX) and peroxidase (PO) (Gonchar et al., 2001). As a chromogen, 3,3′,5,5′-tetramethyl-benzidine dihydrochloride (TMB) was used. The principle of FA determination by AOP-method is based on the measurement of the dye-product accumulation in peroxidative oxidation of chromogen by H_2O_2 (Sibirny et al., 2008) generated from FA in AOX reaction (see reactions 5, 7):

$$\underset{\text{chromogen}}{H_2O_2 + S\text{-}[2H]_{\text{reduced}}} \xrightarrow{PO} \underset{\text{dye}}{2H_2O + S_{ox}} \qquad (7)$$

The analytical parameters of the FdDH-based method have been determined (Fig. 5) in comparison with enzymatic AOP-method and several chemical methods based on the use of Nash's reagent, 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald), chromotropic acid and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH). It was clearly shown, that AOP-method has the highest sensitivity. The slope of the corresponding calibration curve is equal 59.3, corresponding to an apparent millimolar extinction of the formed coloured product (in $mM^{-1}\ cm^{-1}$). Actually, the observed slope value equals the millimolar extinction coefficient (ε_{mM}) multiplied by conversion factor for the enzymatic reaction (k):

$$\text{Slope} = \varepsilon_{mM} \times k \qquad (8)$$

The value of ε_{mM} for the oxidized TMB is equal 81.7 mM^{-1} cm^{-1} (Gonchar et al., 2001), so the conversion coefficient of the analyte for AOP-method at the used experimental conditions is 72.6 %. For the FdDH-based method, conversion factor of the analyte is 32.9 %, assuming a milimolar extinction for NTB-formazane as 10.2 mM^{-1} cm^{-1} at 570 nm in acidic medium.

The linearity of calibration curve for AOP-method is kept even at high optical densities - up to 0.9 which corresponds to 15 µM FA in final reaction mixture (15 nmol ml^{-1}), and the threshold sensitivity of the method is about 0.8 nmol ml^{-1}. These analytical parameters are the best as compared to four chemical methods, even with the use of MBTH or Purpald.

FdDH-based method reveals linearity (at enzymatic conversion above 33 %) at least to 100 µM FA, and its sensitivity is close to Nash's method (the corresponding slopes are 3.36 and 4.46, respectively). Compared to AOP-method, sensitivity of FdDH-based-method is 18-fold less.

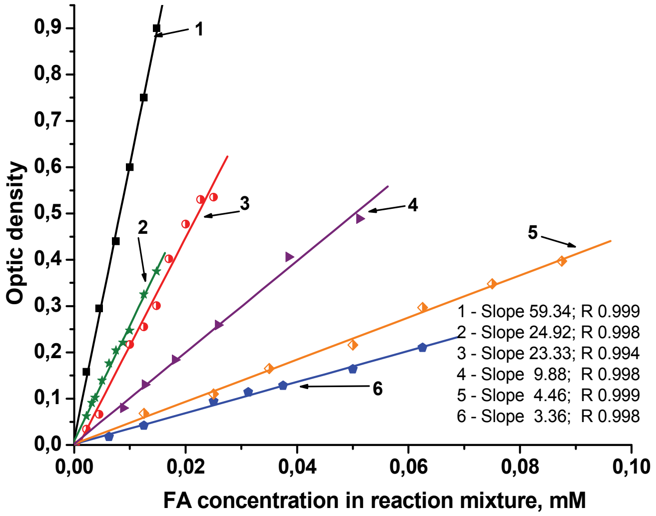


Fig. 5. Comparative analysis of FA-assay methods, using: 1- AOX and PO (AOP); 2 - MBTH; 3 - "Purpald"; 4 -chromotropic acid; 5 - Nash's reagent and 6 - FdDH. The slopes of calibration curves characterize the sensitivity of the methods. Slope values and coefficients of linear regression are shown for each calibration curve.

4.2.2 The reliability of the enzymatic methods for assay of FA in real samples

The FdDH- and AOX-based methods were tested on the real wastewater samples containing FA. We have tested FA in real samples of wastes by the developed enzymatic methods in comparison with standard chemical approaches. It has been demonstrated that in order to evaluate the possible interfering effect of real samples' components on FA assay, it was necessary to perform a standard addition test in both approaches (chemical and enzymatic) and that analytical data obtained by enzymatic method are more reliable than chemical ones.

As shown in Table 4, the comparison of FA concentrations for the FdDH- and AOX-based methods and two routinely used chemical ones (chromotropic acid and MBTH), showed a good correlation between the four approaches. Only in some cases (samples of wastewater DK5 and DK7), with a lower FA content, the difference between the compared methods is higher, than 15 % - 41 % and 26 %, respectively. A relatively high difference is also observed between two chemical methods for the mentioned above samples – 37 % and 21 %. This can be explained by a higher error in measurement of low optical density values obtained for samples with a low FA content. On the other hand, it is worth emphasizing that the chemical approaches used are not free from possible mistakes due to interference from co-impurities, usually present in wastewater samples, for example, phenol, which is an attendant pollutant of FA-containing wastes (Polish standard, 1988).

To evaluate the possible interfering effect of the components of wasterwater samples on FA assay by the FdDH-based and chromotropic acid methods, we used a standard addition test (SAT) for sample WW-A (Table 4, Fig. 6A and B).

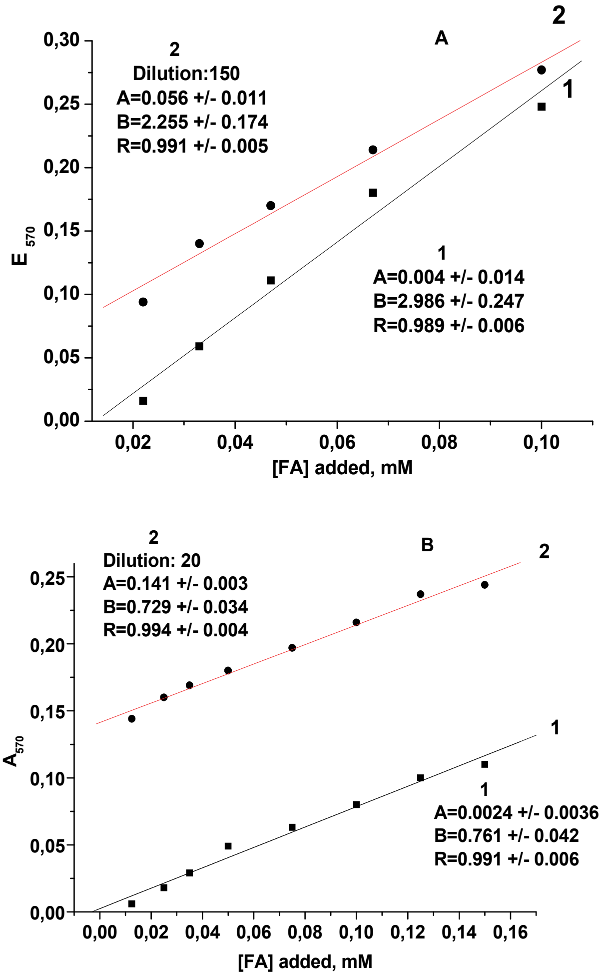


Fig. 6. Standard addition test for the FA assay by the chromotropic acid method (A) and the FdDH-based method (B). Curve 1 corresponds to the calibration experiment performed for FA solutions (traditional calibration), and curve 2 corresponds to the standard addition calibration (FA was added at different concentrations to the diluted wastewater sample; WW-A). Some statistical data are presented on the graphs: parameters of linear regression (coefficients of the equation Y =A+BX, where Y = OD, X = FA concentration (mM), A = OD of the variant without addition of exogenous FA, and B = slope value); R = linear regression coefficient.

Sample/ Method	Enzymatic methods		Chemical methods	
	FdDH-based	AOX-based	Chromotropic acid	MBTH
DK 1	7.89±0.59	9.60±0.45	9.30±0.61	9.56±0.51
DK 2	6.66±0.26	8.12±0.20	8.70±0.50	8.06±0.32
DK 3	6.88±0.41	8.01±0.44	7.20±0.33	7.84±0.36
DK 4	7.58±0.32	6.86±0.9	7.10±0.36	6.30±0.46
DK 5	2.32±0.08	1.97±0.12	1.65±0.35	1.20±0.15
DK 6	5.73±0.32	5.60±0.28	4.64±0.24	4.99±0.06
DK 7	2.47±0.15	2.19±0.2	1.62±0.17	1.96±0.20
WW-A	112±4.5 (SAT) 84.4±6.5 (routine test)	-	116±5.1(SAT) 111±6.1 (routine test)	-

Table 4. Comparison of different methods for FA assay (mg/L) in wastewater samples

As can be seen from Fig. 6, the chromotropic method is more sensitive to the interfering effect of the wasterwater sample components than the enzymatic method: the slope values of the calibration curves obtained for FA in water and in the background of wasterwater sample (WW-A) differed by 24% (2.986 and 2.255, respectively). The respective values obtained for the enzymatic method were 0.761 and 0.729, a difference of only 4.2 %, which is within the limit of statistical deviation. Thus, we can conclude that analytical data obtained by the FdDH-based method are more reliable than the chemical ones. Due to this very important analytical feature of the enzymatic method, it can be recommended for practical application in lieu of chemical methods, which are labour-intensive and time consuming, thereby eliminating the need to distil the samples or perform standard addition test (as in the case of phenol contamination).

The FdDH-based method was tested on different FA-containing vaccines (Paryzhak et al., 2007). As shown in Table 5, the comparison of FA concentration obtained by the FdDH-based method and by two routinely used chemical ones, showed a good correlation between both approaches. Lower levels of FA in anti-diphtheria vaccines, obtained using the enzymatic method as compared to the chemical methods may be due to the inhibitory effect on the enzyme by the Hg-containing compound, 0.01% merthiolate, a vaccine preservative.

Sample/ Method	Enzymatic methods	Chemical methods	
	FdDH-based	Chromotropic acid	MBTH
Anti-diphtheria vaccine	15±2.5	36±1.4	31±2.0
Anti-diphtheria and tetanus vaccine	17±1.9	27±2.0	29±2.7
Polio-vaccine "Imovax"	30±3.0	27±2.6	–
Tetanus vaccine	10.2±0.6	9.0±0.2	12.0±0.2

Table 5. Comparison of FA assay methods (mg/L) in the different vaccines

4.2.3 AOX based method for simultaneous assay of methanol and FA in industrial wastewater

We describe a new enzymo-chemical method for the simultaneous assay of methanol and FA in mixtures, which exploits AOX and aldehyde-selective reagent - 3-methyl-2-benzothiazolinone hydrazone, MBTH (Sibirny et al., 2008). Pre-existing FA content is detected without treating samples by AOX (CD_0 in reaction 9); and methanol content is determined by an increase in colored product concentration due to the methanol-oxidising reaction (CD_Δ in reaction 10).

$$[CH_2O]_0 + MBTH \rightarrow [MBTH\text{-}CH_2O]_0 \xrightarrow[Azine]{FeCl_3} \text{Cyanine dye } (CD_0) \qquad (9)$$

$$(\lambda max=670 \text{ нм})$$

$$CH_3OH + O_2 \xrightarrow{AOX} [CH_2O]_\Delta \xrightarrow{MBTH} [MBTH\text{-}CH_2O]_\Delta \xrightarrow[-H_2O_2]{FeCl_3} \text{Cyanine dye } (CD_\Delta) \qquad (10)$$

Methanol is oxidized to FA by AOX, and FA is oxidized further by AOX. In the presence of MBTH, FA reacts with MBTH, to form an azine adduct that prevents the further enzymatic oxidation of FA by AOX. In this reaction MBTH plays a double role. During the first step of reaction, it forms a colorless azine adduct with pre-existing and enzymatically formed FA, and masks it from further oxidation by AOX, and during the second step of reaction, MBTH facilitates the non-enzymatic oxidation of the azine product to cyanine dye in the presence of ferric ions in acid medium. Pre-existing FA content is assayed by colorimetric reaction with MBTH, without treating samples by AOX, and methanol content is determined by a gain in a colored product due to methanol-oxidising reaction. This enzymo-chemical method of differential detection of FA and methanol in mixtures was used to analyze samples of a commercial product, formalin, which is a concentrated FA solution containing methanol as a stabilizer that inhibits FA polymerization. The results of this analysis, shown in Table 6, are in a good agreement with the data obtained by traditional chemical methods and gas-chromatography.

Sample	Methanol (MeOH) and formaldehyde (FA) content, % (M±m, n=4)				
	AOX-chemical method		Gas-chromatography	Chemical method (chromotropic acid, permanganate)	
	MeOH	FA	MeOH	MeOH	FA
I	2.59 ± 0.19	4.36 ± 0.23	3.3 ±0.5	2.7 ±0.13	4.62 ±0.11
II	4.61± 0.34	7.15 ± 0.37	5.39 ± 0.5	4.72 ± 0.27	7.27 ± 0.2
III	3.29 ±0.38	6.95 ± 0.23	3.4 ± 0.5	3.01 ± 0.08	6.49 ± 0.28
IV	2.8 ± 0.32	6.23 ± 0.25	3.53 ± 0.5	2.70 ± 0.05	6.58 ± 0.33
V	0	1.72 ± 0.2	0	0	1.85 ± 0.1
VI	0	1.48 ± 0.13	0	0	1.73 ± 0.08
VII	3.77 ± 0.30	2.66 ± 0.16	3.13 ± 0.2	3.79 ± 0.12	3.82 ± 0.15
VIII	4.15 ± 0.32	2.14 ± 0.27	3.06 ± 0.5	2.93 ± 0.31	4.11 ± 0.13

Table 6. Results of enzymo-chemical assay of methanol and FA in distillate of wastewaters (compared with the reference methods)

The threshold sensitivity of the assay method for both analytes is near 1 µM which corresponds to 30-32 ng analyte in 1 ml of reaction mixture and is 3.2-fold higher when compared to the chemical method using permanganate and chromotropic acid. The linearity of the calibration curve is reliable ($p < 0.0001$) and standard deviation for parallel measurements of test samples does not exceed 7%. The proposed method, in contrast to the standard chemical approach, does not need the use of aggressive chemicals (concentrated sulfuric, phosphoric, chromotropic acids, permanganate), it is easier to perform, and can be used for industrial waste verification and certification of formaline-containing materials.

4.2.4 AOX- and FdDH-based methods for FA assay in fish food products

Fish products are an important source of food protein. The fish species *Gadidae* are second only to *Clupeidae* in the size of industrial catch, but are preferred as food products whereas *Clupeidae* are more frequently used in agriculture and industry. The tissues of the *Gadidae* fish under inappropriate storage, that is, at non-deep freezing conditions (t°>–30 °C), accumulate highly toxic concentrations of FA due to endogenous metabolic reactions, involving namely the natural osmoprotectant trimethylamine-N-oxide, which acts as antifreeze (Reihbein, 1995). Generated FA can cause the fish to spoil, and even make it dangerous for human health if consumed. These two important reasons highlight the necessity for selective, sensitive and reproducible method to control the content of this dangerous metabolite in some fish products.

The applicability of both enzymes simultaneously used, AOX with peroxidase (AOP-method) and FdDH for FA assay in fish products was demonstrated. Test samples of frozen fish of the *Gadidae* family (hake and cod), most frequently sold in European markets, as well as freshly-killed carp were used. The optimal protocols for obtaining of protein-free extracts and for testing procedures have been elaborated (Pavlishko et al., 2003). The analytical parameters of both enzymatic methods have been determined in comparison with several chemical methods based on the use of Nash's reagent, Purpald, chromotropic acid and MBTH. Fig. 5 presents calibration curves for the two enzymatic methods and compares them with the best of the chemical methods. It is clearly shown, the AOP-method has the highest sensitivity.

The FdDH-based method is nearly 18-fold less sensitive, compared to AOP-method, because of a lower molar extinction of the corresponding formazane: the analyte conversion factor is 32.9 %, assuming a milimolar extinction for NTB-formazane as 10.2 mM^{-1} cm^{-1} at 570 nm in acidic medium (own data). FdDH-based method sensitivity is close to Nash's method (the corresponding slopes are 3.36 and 4.46, respectively). Linearity of FdDH-based method is at least to 100 µM FA.

There was a good correlation between the analytical results of both enzymatic methods as compared with chemical approaches, though AOX-based assay is preferred due to its higher sensitivity, good linearity, insensitivity to the interference by test sample contaminants and the usage of non-aggressive reagents for the sample pre-treatment and assay procedure (Table 7 and Table 8).

Table 8 shows FA concentrations as measured by all of the tested methods. To compare the validity of both enzymatic methods, and to evaluate possible interference by the chemical background of the test samples on analytical results, FA content was analyzed using in fish protein-free extracts using a routine method (with an external calibration) as well as a multiple standard addition test (MSAT). Simultaneously, FA concentration was also analyzed by two chemical methods, using chromotropic acid and MBTH.

Fish Approach	Hake	Cod	Carp
AOX- based, M±m	90.0±2.6	74.1±1.1	0
Nash's, M±m	121.6±0.9	96.8±3.1	0
Purpald, M±m	100.6±1.6	59.7±3.1	0

Table 7. Results of FA assay (in mg per 1 kg of wet weight of muscle tissue) in protein-free extracts of fish using three independent approaches: AOX- based method, Nash's and Purpald methods

Method	Multiple standard addition test	Routine test
FdDH- based, M±m	101.8±3.2 ($p<0.05$)*	64.3±8.6
AOX- based, M±m	95.3±3.7 ($p>0.05$)**	98.0±3.5
MBTH, M±m	104.3±5.6 ($p>0.05$)**	106.4±7.9
Chromotropic acid, M±m	100.5±1.2 ($p>0.05$)**	102.8±7.3

**Difference between routine test and MSAT is statistically significant; **Difference between routine test and MSAT is statistically insignificant.*

Table 8. Results of FA assay (in mg FA per kg of wet weight of muscle tissue) in protein-free extract of the fish hake, using three independent approaches: FdDH-metod, MBTH and chromotropic acid.

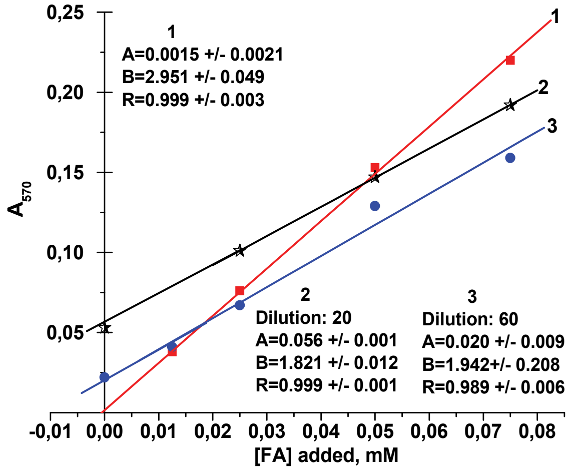


Fig. 7. Multiple standard addition test for FA assay in hake, using the FdDH-based method. Curve 1 corresponds to the calibration experiment performed for aqueous solutions of FA (external traditional calibration), and curves 2 and 3 correspond to the multiple standard addition test (FA was added at different concentrations to the diluted real sample). Some statistical data are presented on the graphs: parameters of linear regression (coefficients of the equation Y =A+BX, where Y - optical density, X - FA concentration (mM), A – optical density for the sample without addition of exogenous FA, and B - slope value); R - linear regression coefficient

It was demonstrated that some fish products (hake and cod) contain high FA concentrations, up to 100 mg/kg wet weight, while FA content in carp was negligible.

The slopes of the calibration curves prepared on fish extracts are dependent upon the dilution factor, a bigger dilution results in a higher the slope (meaning there is less of an interfering effect). For the external calibration (that has no test sample background, and corresponds to an infinite dilution), the slope is the highest, 2.95 as compared to 1.94 (a 60-fold dilution of the test sample) and 1.82 (20-fold dilution of the sample). For the AOP and chemical methods, there is no significant difference between routine and MSAT-variants of the assay.

As shown in Table 8, there is a good correlation between all analytical data obtained in the MSAT-variant of analysis, which was not the case for the results obtained by the routine variant of analysis with external calibration. This may be due to the interference of some components which are co-extracted by TCA from the fish tissue. This suggestion is clearly supported by the data obtained by the FdDH-based method (Fig. 7).

5. Construction and investigation of FA-selective biosensors

5.1 AOX- based enzymatic and microbial sensors

For the quantitative analysis of FA there have been developed potentiometric biosensors using whole cells of mutant strains of methylotrophic yeasts and AOX as the biorecognition elements and pH-Sensitive Field Effect Transistors (pH-SFETs) as a transducer. As an analytical signal in the pH-SFET-based sensor, the production of protons due to FA conversion into formic acid was exploited.

To develop cell-based FA - sensitive potentiometric sensor (Korpan et al., 1993), the mutant strain *H. polymorpha* A3-11 with repressed activities of AOX and formate dehydrogenase and blocked activity of formaldehyde reductase was obtained. The biosensor demonstrated high specificity/selectivity to FA with no response to several organic acids, methanol and other alcohols, except for the very low sensitivity to ethanol. The linear dynamic range of the sensor's response corresponds to FA concentration of 2 to 200 mM.

Partially purified AOX preparations have also been used as recognition elements of pH-SFET-based potentiometric sensor selective to FA (Korpan et al., 1997; Korpan et al., 2000). The response time in steady-state measurement mode is in the range of 10–60 s, but if measured in kinetic mode the response time of the created biosensors was less than 5 s. The linear dynamic range of the sensor output signals corresponds to 5–200 mM of FA concentration. It was quite suprisingly that AOX-based sensors gave no signal to methanol and was highly selective to FA. These results seem rather unusual because methanol is the preferred substrate for most AOX's, being directly oxidised to FA. The absence of a measurable response to methanol may be explained as follows: a) the rate of methanol oxidation in AOX reaction is about 10-fold higher than that of FA; b) effective oxidation of methanol is likely to result in the local oxygen depletion in the bioactive zone limiting the oxygen available for subsequent FA oxidation; c) FA produced from methanol can diffuse from the bioactive zone back into the bulk solution without oxidation; d) FA, being very reactive, is likely to bind covalently with NH_2-groups of AOX.

All these factors may result in a decrease of the concentration of formic acid produced from methanol in bioactive membranes to a level lower than the sensitivity of the potentiometric biosensor described and therefore no response to methanol is apparent. It is noteworthy that most of the described factors do not work for intact yeast cells where FA and methanol are oxidised in different reactions.

It should be noted that contrary to other pH-SFET-sensors, the signal of AOX-based sensors (Korpan et al., 2000) to FA is not repressed, but even enhanced in the presence of Tris-HCl buffer. The chemical nature of this effect seems to be the reaction of FA with aminogroup of tris(hydroxymethyl)aminomethane with production of a hydroxymethylamine derivative, which is a weaker base compared to the parent compound and this reaction results in releasing free protons. This unexpected effect is the first reported example of specific "chemical enhancement" of the pH-SFET biosensor response.

A highly stable and sensitive amperometric bi-enzyme biosensor (Smutok et al., 2006) was developed for assay of ethanol, as well as of FA, using the highly-purified AOX preparation (Shleev et al., 2006), isolated from the yeast cells of *H. polymorpha* C-105. The sensor's layer was created with a non-manual electrochemically-induced immobilization procedure using a new type of Os-complex modified electrodeposition paints (EDP) for horseradish peroxidase placing in a first layer and a cathodic EDP for AOX immobilization and stabilization in a second layer. The used redox EDP assures fast electron transfer between the integrated peroxidase and the electrode surface at a low working potential.

Bioanalytical properties of an optimized biosensor such as response time, dynamic range for different analytes (FA and alcohols), operational and storage stability were investigated. The obtained sensors showed significantly improved stability as compared to previously reported sensors based on AOX. But such biosensor can be used for FA assay in wastes water only in the absence of aliphatic alcohols in tested probes.

For amperometric assay of FA, permeabilized and intact cells of the mutant strain *H. polymorpha* C-105 with a high activity of AOX as the biorecognition elements, were tested. Different approaches were used for monitoring FA-dependent cell response including analysis of their oxygen consumption rate by the use of a Clark electrode, as well as of oxidation of redox mediator at a screen-printed platinum electrode covered by cells entrapped in Ca-alginate gel. It was shown that oxygen consumption rate of permeabilized cells reached its saturation at 4 mM of FA (23 ^{o}C). The detection limit is 0.27 mM. In the presence of redox mediator 2,6-dichlorophenolindophenol (DCIP), the screen-printed platinum band electrode covered by permeabilized cells did not show any current output to FA. In contrast, well-pronounced amperometric response to FA was observed in the case of intact yeast cells in the presence of DCIP. However, intact cells did not show a strict substrate selectivity, because of functioning of the whole electron transport chain. In contrast, essentially improved substrate selectivity was observed in the case of permeabilized cells where only AOX is responsible for the oxygen consumption. Obviously, it is necessary to perform a directed metabolic engineering of the yeast cells to improve their bioanalytical characteristics in the corresponding biosensors (Khlupova et al., 2007).

5.2 FdDH-based capacitance, impedance and conductometric biosensors

Recombinant yeast FdDH (Demkiv et al., 2007) was used as a FA-recognising element coupled with semiconductor-based structure $Si/SiO_2/Si_3N_4$ as a transducer (Ben Ali et al., 2007). The bio-recognition element had a bi-layer architecture and consisted of FdDH, cross-linked with albumin, and two cofactors (NAD^+ and GSH) in the high concentrations (first layer); the second layer was a negatively charged Nafion membrane which prevented a leakage of negatively charged cofactors from the bio-membrane. Changes in capacitance properties of the bio-recognition membrane were used for monitoring FA concentration in a bulk solution. It has been shown that FA can be detected within a concentration range from 10 μM to 25 mM with a detection limit of 10 μM (Fig. 8 and Table 9).

A similar FA sensitive biosensor based on commercial preparation of bacterial FdDH as recognizing element has been also described (Ben Ali et al., 2006). As transducers, gold electrodes $SiO_2/Si/SiO_2/Ti/Au$ and electrolyte- insulator-semiconductor Si/SiO_2 structures (EIS) have been used. Changes in capacitance and impedance properties of the bio-recognition membrane have been used for FA monitoring, It has been shown that FA can be detected within a concentration range from 1 μM to 20 mM depending on the used type of transduction, with a detection limit 1 or 100 μM for gold- and EIS-based transducers, respectively.

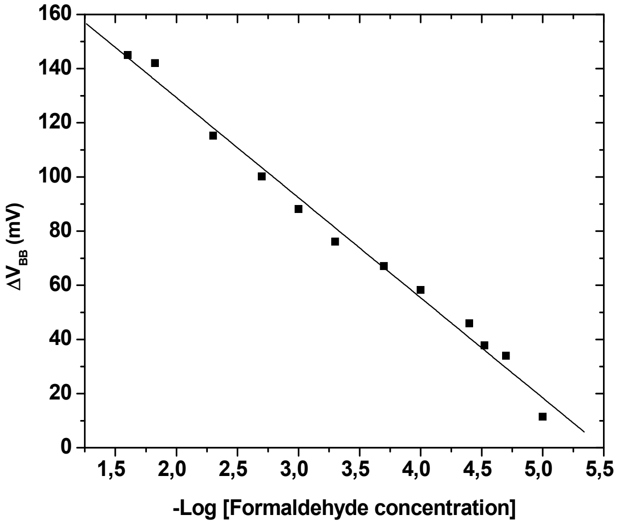


Fig. 8. Response of bio-functionalized $Si/SiO_2/Si_3N_4$ structure for FA (in logarithms of the molar concentration) in the tested solution

To construct FA-selective conductometric biosensor, the novel method was proposed using thin-film interdigitated gold planar electrodes and FdDH (commercial bacterial or recombinant yeast rFdDH) co-immobilized with cofactor(s) in the presence of DEAE-Dextran (Korpan et al., 2010).

DEAE-Dextran prevents active leakage of cofactors out of the bioselective layer and allows for the reuse of the biosensor without the regeneration of the cofactor(s), since its(their) concentration(s) in the layer is (are) high, constituting 100 mM and 20 mM for NAD^+ and GSH, respectively.

The working signals obtained for the conductometric biosensors based on rFdDH and FdDH, allow to assume that the apparent value of pH shift in the bioselective membrane does not exceed 0.4 units of pH. Due to the pH of the working solution (exactly, 8.7) used for measurements and some processes of protons masking, the steady-state value of protons inside the selective membrane can be estimated as 10^{-7} M. This means that during one assay the concentration of cofactor (NAD^+) consumed during FA transformation is about 6 orders lower in comparison with the concentration of NAD^+ (10^{-1} M) immobilized on the transducer surface. Even taking into account that 90% of NAD^+ could leak out of the membrane due to the washing procedures, and assuming that transformation efficiency of the analyte is approx. 1%, the remaining effective level of NAD+ has to be quite enough to perform up to 1.000 measurements using the same transducer. These calculations correlate well with the data obtained experimentally by tests performed on the developed biosensors over a 3 month period: more than 200 measurements on the same transducer have been made without any loss of the sensor signal and sensitivity. At the same time, most of the previously reported FdDH-based sensors (Vianello et al., 1996; Kataky et al., 2002) are based on the usage of covalently bounded NAD+, which essentially decreases its availability and effectiveness for enzyme-substrate interactions.

It was studied the dependence of the developed rFdDH-based conductometric biosensor response on FA concentrations (Table 9). It has been shown (Fig. 9), that the conductometric biosensor is highly selective and even specific to FA, the basic substrate of FdDH. Other compounds, in particularly ethanol and ethanol, caused the significant changes in responces of the proposed conductometric biosensor. However, the usage of methanol and ethanol mixtures with FA leads to slight alteration of rFdDH-based conductometric sensor response values toward FA.

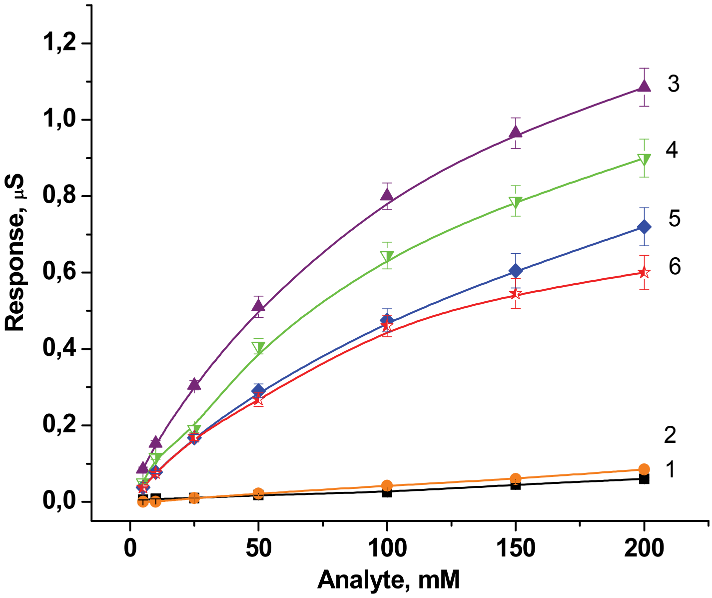


Fig. 9. Selectivity of the developed rFdDH-based biosensor to different substrates: 1 – methanol, 2 – ethanol, 3 – formaldehyde, 4 - equimolar mixture of FA and methanol, 5 - equimolar mixture of FA and ethanol, 6 – methanol, ethanol and FA. The measurements were performed in 10 mM borate buffer, pH 8.7

5.3 FdDH- and cells-based amperometric biosensors

The recombinant yeast cells Tf 11-6 *H. polymorpha* and FdDH isolated from these cells were used as biorecognition elements of amperometric biosensors. In the physiological electron-transfer pathway, electrons are transferred from FA via intermediate G-SH to the active centre of FdDH with the simultaneous reduction of NAD+ to NADH. To design an electron-transfer pathway for the immobilised FdDH as a bioselective element of the sensor, the enzymatically generated NADH has to be re-oxidised additionally at the electrode surface using a suitable redox mediator (Fig. 10).

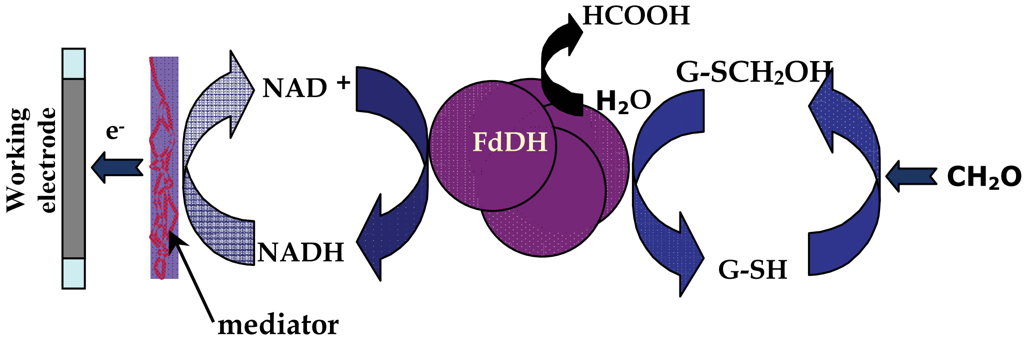


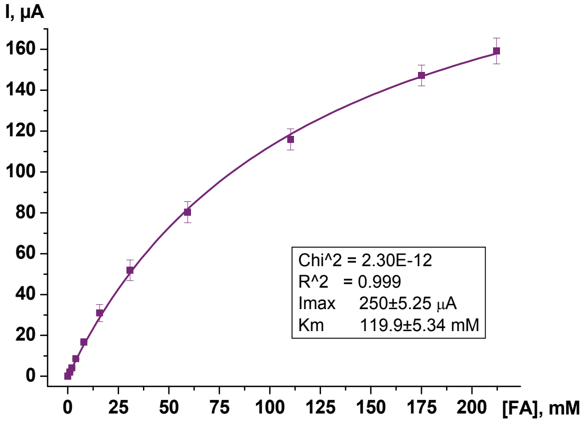
Fig. 10. Schematic representation of the electron pathway for FA detection by FdDH-based amperometric biosensor

The best mediators for enzyme biosensors were shown to be positively charged cathodic electrodeposition paints modified with Os-bis-N,N-(2,2′-bipyridil)-dichloride ($[Os(bpy)_2Cl_2]$) complexes (Ngounou et al., 2004). Among five tested Os-containing redox polymers of different chemical structures and properties, complex *1CPOs* of osmium-modified poly(4-vinylpyridine) with molecular mass of 60 kDa and containing diaminopropyl groups was selected as the most suitable redox polymer. At the same time, the polymer layer served as a matrix for keeping the negatively charged low-molecular cofactors, GSH and NAD^+, within the bioactive layer. In cells-based biosensors phenazine methosulfate (free-diffusing redox mediator, PMS) exhibited the best electron transfer characteristics (Demkiv et al., 2008; Gayda et al., 2008).

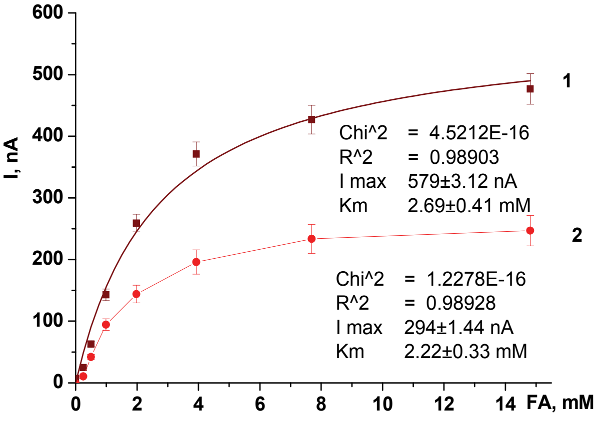
For construction of the envisaged FA biosensor, it was proposed a sophisticated sensor architecture with the aim of securely fixing all sensor components in a bioactive layer onto the transducer surface. Especially, the sensor architecture was designed to prevent any leakage of the low-molecular and free-diffusing cofactors of the enzyme, thus enabling FA determination without the addition of the cofactors to the analyte solution [(Demkiv et al., 2008; Gayda et al., 2008). In the optimized biosensor's construction, platinised graphite electrode, with 3.05 mm diameter, was used as a transducer and $[O_s(Me2bpy)_2Cl_2]$-modified positively charged cathodic paint *1CPOs* was found to be the best redox mediator, as well as a good matrix for enzyme or cells electrodeposition and for holding enzyme's co-factors, GSH and NAD, in a bioactive layer. Covering of the bio-layer by a negatively charged Nafion membrane additionally prevented the leakage of cofactors and enhanced sensor's stability.

Bioanalytical characteristics of the constructed biosensors were studied in detail: kinetics, dynamic and linear range, selectivity, and effect of temperature on sensor output (Table 9). For the enzyme-based biosensor, the maximum current value was 250 ± 5.25 µA and the apparent Michaelis-Menten constant (K_M^{app}), derived from the FA calibration curves, was 120 ± 5.3 mM with a linear detection range for FA up to 20 mM. For the cell-based biosensor, the maximum current value was 1.07 ± 0.04 µA, and K_M^{app} was 20.1 ± 1 mM with a linear detection range for FA up to 8 mM. The optimal pH-value for the developed biosensors was in the range of 7.6 to 8.3 with an optimal temperature between 45-50 °C (Gayda et al., 2008), due to a higher thermostability of the enzyme used, and thermotolerance of recombinant yeast cells. The bioanalytical properties of the developed biosensors were evaluated with the specific aim of improving the long-term operational stability of the sensor. The novel biosensors demonstrated a good sensitivity, high selectivity to FA and a good stability (Fig. 11-13).

A typical response of the developed *1CPOs-NAD*$^+$*-FdDH*-modified electrodes towards FA is shown in Fig. 11 A. As can be seen from Fig. 11 B, K_M values for FA, obtained by using intact- and permeabilized recombinant cells-based biosensors, are similar, but I $_{max}$ value for the intact-cell-based sensor is 2-fold higher than for the sensor with permeabilized cells.



(A)



(B)

Fig. 11. Chronoamperometric determination of FA, using A - *1CPOs-NAD*$^+$*-FdDH-GSH-*Nafion -based biosensor; B - *Cells*-Tf *11-6*-NAD+-GSH-Nafion-modified graphite electrode (1) and perm*Cells*-Tf *11-6*-NAD$^+$-GSH-Nafion-modified graphite electrode (2). Potential 0 mV against Ag/AgCl/3M KCl in phosphate buffer, pH 8.0, with 0.2 mM PMS

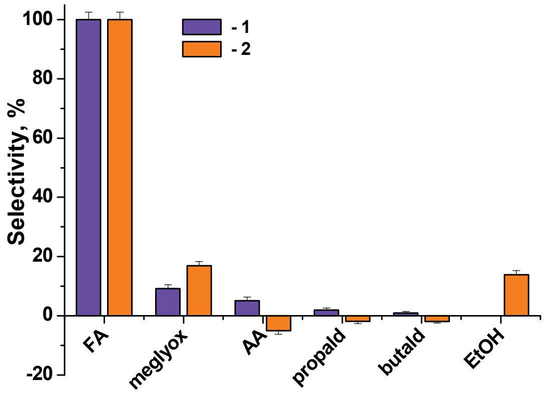


Fig. 12. Selectivity of the developed amperometric enzyme-(1) and cell-based (2) biosensors to different substrates: meglyox – methylglyoxal; AA – acetaldehyde; propald – propionaldehyde; butald – butyraldehyde; EtOH – ethanol

Another amperometric biosensor, developed (Nikitina et al., 2007) in a co-operation with the scientific group headed by Prof. E. Czoregi (University of Lund, Sweden), was a bilayer bi-enzyme sensor based on diaphorase and FdDH, together with [Os(4,4'-dimethylbipyridine)$_2$Cl]/(PVP-Os). The sensor's architecture was comprised of a first layer containing diaphorase from *Bacillus stearothermophilus,* cross-linked with the PVP-Os redox polymer. On the top of this layer, a second layer was formed by additional cross-linking of FdDH with poly(ethylene glycol)(400)diglycidyl ether. The sensor architecture was optimised with respect to efficient electron transfer and stability of the enzyme(s). The developed bi-enzyme FdDH-DPH-PVP-Os-modified electrode displayed the best characteristics for FA detection in flow injection mode of a sample.

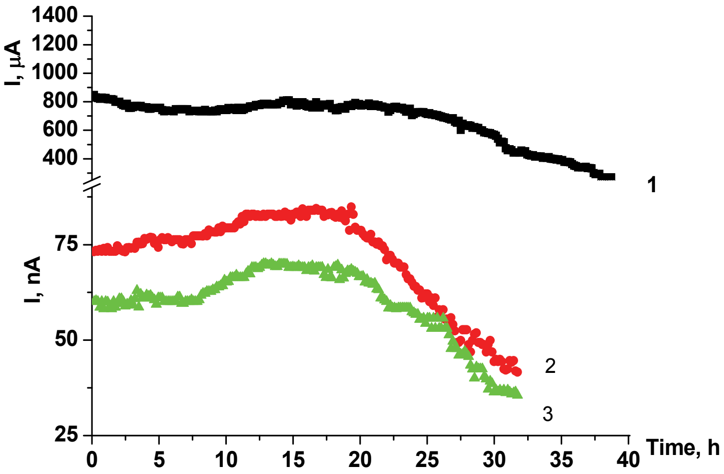


Fig. 13. Operational stability of the optimised FA biosensors: enzymatic-based (1), cell-based (2) and permeabilized cell-based (3). Bioelectrodes were tested in an automatic sequential injection analyser. Flow-rate 5 ml min^{-1}; sample injection every 4 min. Potential 0 mV against Ag/AgCl/3M KCl in phosphate buffer, pH 8.0, with 0.2 mM PMS

The basic bioanalytical characteristics of the bi-enzyme biosensor, polarized at +180 mV vs. NHE, are presented in Table 9 and Fig. 14. The biosensor-FA reaction obeys typical Michaelis-Menten kinetics. The detection limit was found to be 32 µM, while the dynamic range was shown to be linear between 0.05 and 0.5 mM FA. The slope of the calibration curve (sensitivity) and the linear correlation coefficient were 22 $Am^{-2}M^{-1}$ and 0.998, respectively. The stability of the FdDH immobilized on the electrode was also evaluated. When the biosensors were stored at 4 ^0C in phosphate buffer, pH 7.5, the response was linear with a loss of 50% of the activity after 24 h. Dry storage of the immobilized electrode at the same temperature resulted in the complete inactivation of the immobilized enzyme.

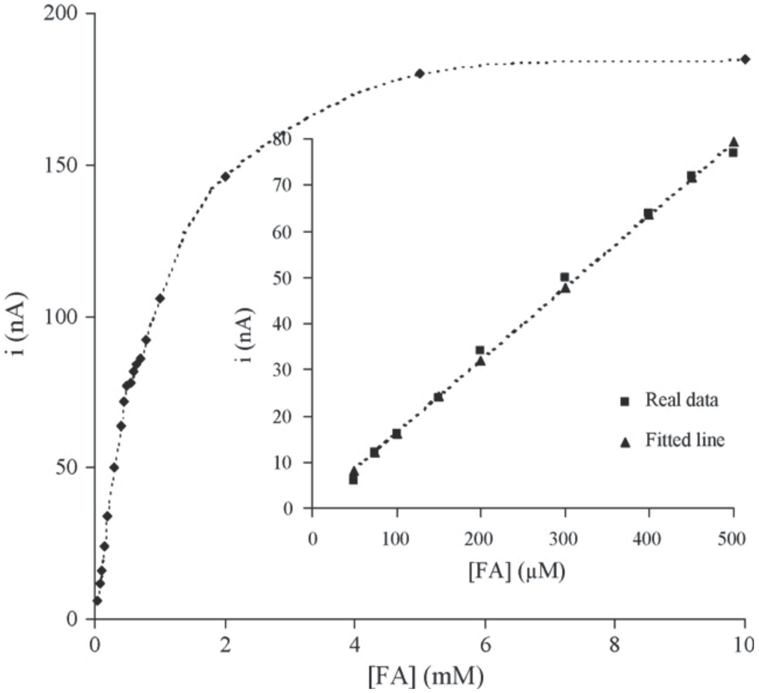


Fig. 14. Calibration curve of the FdDH-DPH-PVP-Os-modified electrode (0.5 mM NAD^+; 0.25 mM GSH; 0.1 M phosphate buffer, pH 7.5; *E* appl = 160 mV; 0.4 ml/min flow rate)

5.4 The comparison of the developed FA-selective biosensors

Tables 9 and 10 represent a brief summary of the published results on the developed microbial and enzyme-based FA biosensors with differend types of signal detection. The amperometric biosensors, enzyme- and cell-based, work at a very low applied potential, compared with other known biosensors (zero or 160 vs. 340, 610 or 560 mV), thus the possible interferences (*e.g.*, methanol, ethanol, acetic acid) should be considerably reduced. Different approaches were used for biosensor monitoring FA-dependent cell response: 1) analysis of their oxygen consumption rate by using a Clark electrode; 2) assay of oxidation of redox mediator at a screen-printed platinum electrode covered by cells entrapped in Ca-alginate gel (Khlupova et al., 2007).

The dynamic ranges of all described biosensors were of micromolar values. As can be seen from Tables 9 and 10, AOX- and FdDH-based biosensors, constructed for potentiometric and conductometric signals registration, have high storage stability.

Characteristics	FdDH-based				AOX-based	
	Bi-enzyme	Mono-enzyme			Mono-enzyme	Bi-enzyme
Type of signal detection	Amperometric		Capacitance	Conductometric	Potentiometric	Amperometric
Detection limit, mM	0.032	0.003	0.01	10	-	0.024
Linear range, mM	0.05-0.5	up to 20.0	0.01-25	10-200	5-200	4
I_{max}, µA	0.18	250	-	-	-	0.8
Sensitivity, $*A \cdot m^{-2} \cdot M^{-1}$	22*	358*	31 mV/ decade	-	50 mV/ decade	114*
Storage stability, days	1	3	-	140	120	14
Reference	Nikitina et al., 2007	Demkiv et al., 2008	Ben Ali et al., 2007	Korpan et al., 2010	Korpan et al., 2000	Smutok et al., 2006

Table 9. Bioanalytical characteristics of enzyme-based biosensors

Parameter	Cells *H. polymorpha* C-105				Cells *H. polymorpha* Tf 11-6		
	Intact		Permeabilized		Intact		Permeabilized
Applied potential (mV)	-600	+200	-600	-	+200	0	0
Mediator	-	DCIP	-	-	*CP58-Os*	PMS	PMS
Registration type	Clark electrode	Amperometric	Clark electrode	Potentiometric	Amperometric		
Linear dynamic range, mM	up to 3.0	1.0-7.0	0.3-4.0	5-50	0.5-6.0	0.25-8.0	1.0-2.5
Detection limit, mM	0.6	0.74	0.27	3.5	0.003	0.11	0.5
Sensitivity	*1.15	8.62 $nA \cdot mM^{-1}$	*0.44	-	2.65 $\mu A\ mM^{-1}$	37.5 $nA \cdot mM^{-1}$	-
Storage stability	-	-	-	30	16	20	7
Reference	Khlupova *et al.*, 2007			Korpan *et al.*, 2000	Demkiv *et al.*, 2008, Paryzhak *et al.*, 2008		

* Oxygen consumption rate per 1 mM of FA ($\mu M\ O_2 s^{-1} \cdot mM^{-1}$)

Table 10. Comparison of microbial (yeast cells-based) FA-sensitive biosensors. DCIP - 2,6-dichlorophenolindophenol; PMS - phenazine methosulfate

Such excellent stability is intrinsic for cell-based sensors, too. Both amperometric and capacitance biosensors, AOX-, FdDH- and cells Tf 11-6 based, are very sensitive to low FA concentrations (Demkiv et al., 2008, Smutok et al., 2006, Ben Ali et al., 2007). FdDH-based biosensors have very important property for FA analysis in real samples – high selectivity to FA, compared with AOX-and cells-based sensors (Gayda et al., 2008).

5.5 Application of biosensors for FA-monitoring in real samples

The purified FdDH, as well as recombinant *H. polymorpha* cells overproducing this enzyme were used for construction of enzyme-based and microbial electrochemical biosensors selective to FA. The reliability of the developed analytical approaches was tested on real samples of wastewaters, pharmaceuticals, and FA-containing industrial products. As we can see from table 11, the proposed methods, approved on the real FA-containing samples, are well correlated with the results of the known chemical methods and novel FdDH-based analytical kit "Formatest" (Demkiv et al., 2009).

The constructed amperometric biosensors revealed a high selectivity to FA (100 %) and a very low cross-sensitivity to other structurally similar substances: butyraldehyde (0,93%), propionaldehyde (1,89%), acetaldehyde (5,1%), methylglyoxal (9,12%) (Paryzhak et al., 2007). These sensors were applied for FA testing in some industrial goods: Formidron, Descoton forte, formalin and rabbit vaccine against viral hemorrhage. A good correlation was observed between the data of FA testing (Table 11) by the amperometric biosenor's approaches (FdDH and cells-based), proposed enzymatic method "Formatest" and standard chemical methods.

Sample/ Method	Chemical methods			FdDH-based methods				
	MBTH	Chromotropic acid	Purpald	Formatest	Biosensors			
					Amperometric			Conductometric
					FdDH	FdDH*	Cells	FdDH
Formidron	1.64± 0.61	1.48±0.26	1.20 ± 0.20	1.53± 0.31	1.57± 0.13	1.50 ± 0.60	1.48± 0.06	1.69±0.13
Descoton forte	3.57± 0.30	3.59±0.44	3.30 ± 0.30	3.25± 0.80	3.61± 0.13	3.50 ± 0.30	3.29± 0.12	14.10±0.80
Formalin	12.6± 0.73	14.0±0.81	12.9± 0.70	13.5± 0.54	13.6± 0.6	13.6± 0.6	13.8± 0.54	12.99±0.18
Rabbit vaccine against viral hemorrhage	0.038± 0.003	0.029±0.005	0.043± 0.005	0.042± 0.004	0.041± 0.005	-	0.042± 0.002	-
Reference	Demkiv, et al., 2008, Demkiv, et al., 2009				Demkiv, et al., 2008			Korpan et al., 2010

Table 11. FA content in molar concentration in real samples, M±m, determined by different methods: chemical (MBTH, Chromotropic acid); enzymatic method "Formatest", FdDH-based, and biosensor approaches (FdDH- and recombinant cells Tf 11-6 -based). *FdDH - enzyme was Integrated in analyzer "OLGA" with Flow Injection mode.

The conductometric sensors, FdDH- and rFdDH-based (Korpan et al., 2010), were evaluated in determining the FA content in real samples of the industrial product Formalin and two pharmaceuticals, the antimicrobial agent Descoton forte and antiperspirant Formidron, and the results of these tests are summarized in Table 11. As for the amperometric rFdDH-based sensor, the maximal interfering effect for the proposed conductometric biosensors was observed for Descoton, less for Formidron, and the smallest for Formalin. The results obtained for Descoton are due to the presence in this preparation of high quantities of glutaric aldehyde, which consequently changing substantially the mechanical and catalytic properties of the bioselective layer, since it can cause cross-linking reactions. For all investigated samples, a good correlation was observed between the conductometric sensor values and enzymatic or chemical methods. These analytical data confirm the possibility to exploit the developed biosensors for FA assay at least in real samples of non-complicated compositions such as pharmaceuticals, potable water and wastewater.

6. FA removal from indoor air

For removal of FA from indoor air a number of methods have been proposed. Physical adsorption of FA with activated carbon (Boonamnuayvitaya et al., 2005; Tseng et al., 2003), by various fractions of karamatsu bark (Takano et al., 2008) and by zeolites (Cazorla & Grutzeck, 2006) was shown to demonstrate good to high results, but simple adsorption cannot provide a radical solution to the problem, since FA does not decompose, but is only transferred from one phase (air) to another (solid). Efforts, attempting to carry out the physical decomposition of FA, with the help of photo-catalytic, negative ions and ozone air cleaners resulted in the elimination of only up to 50% FA, and failed to reach acceptable FA concentrations as specified by WHO guidelines (0.08 ppm) (Tseng et al, 2003). Chemical decomposition of FA by composite silica particles functionalized with amine groups and platinum nanoparticles demonstrated a very high capacity for removing FA (Lee et al., 2008), but this process is expensive. Another approach to the chemical elimination of FA from air was developed in the work of Sekine, where manganese dioxide was shown to be effective in the oxidation of FA (Sekine, 2002; Tian & He, 2009). Combustion of a formaldehyde-methanol mixture in an air stream on Mn/Al_2O_3 and $Pd\text{-}Mn/Al_2O_3$ catalysts was shown to result in a total conversion of organic compounds (Álvarez-Galván, et al., 2004). Some chemical approachs to FA decomposition are highly effective, but solid wastes still remain as a by-product of these processes, in most cases containing harmful toxic components that cause subsequent utilization problems.

FA removal from air using biological decomposition is still not well developed. Theoretically, biofilters containing natural microorganisms capable of decomposing FA can be used for this purpose. Several biofilters and biotrickling filters were tested for the treatment of a mixture of formaldehyde and methanol (Prado et al., 2004, 2006), and a maximum FA elimination capacity of 180 g m^{-3} h^{-1} (3 μmoles g^{-1} h^{-1}) was reached.

Recently, enzyme-based approaches have been proposed for FA bioremediation of indoor air. To this aim, continuous flow bioreactors based on the immobilized FA-oxidizing enzyme AOX or mutant yeast cells overproducing this enzyme were constructed (Sigawi et al., 2010).

AOX isolated from mutant *H. polymorpha* C-105 cells was immobilized in calcium alginate beads and applied for the bioconversion of airborne FA. The AOX preparation had a specific activity in the range of 6-8 $U{\cdot}mg^{-1}$ protein and was shown to preserve 85-90% of the initial activity after incorporation into the calcium alginate gel. This activity was proven to remain unchanged for up to seven months upon storage of the immobilized enzyme at 4°C.

A fluidized bed bioreactor (FBBR) based on glass columns was filled with gel beads containing immobilized AOX and suspended in phosphate buffer-saline. Columns filled with gel alone were used as control. FA-containing air was bubbled through the columns from the bottom to the top (Fig. 15) as described previously in Sigawi et al, 2010. The results showed that in the case of the 20 ml reactors, the outlet FA concentration was less than 0.03 ppm, i.e. ten-fold less than the threshold limit value (TVL), and the 750 ml reactor outlet air contained no FA at all. The FA concentration in the gas phase at the outlet from the control columns without the enzyme was essentially higher (0.09-0.1 ppm) than the test columns, but also relatively low compared to the input level, evidently due to FA dissolution in the liquid phase of the column and possibly also due to adsorption by the gel. The FA concentration in the bioreactor liquid phase of the test column was ca. 1-2 mM (Fig. 16), and in the control experiment ranged from 6 mM (750 ml reactor, Fig. 16) to 20 mM (20 ml reactor).

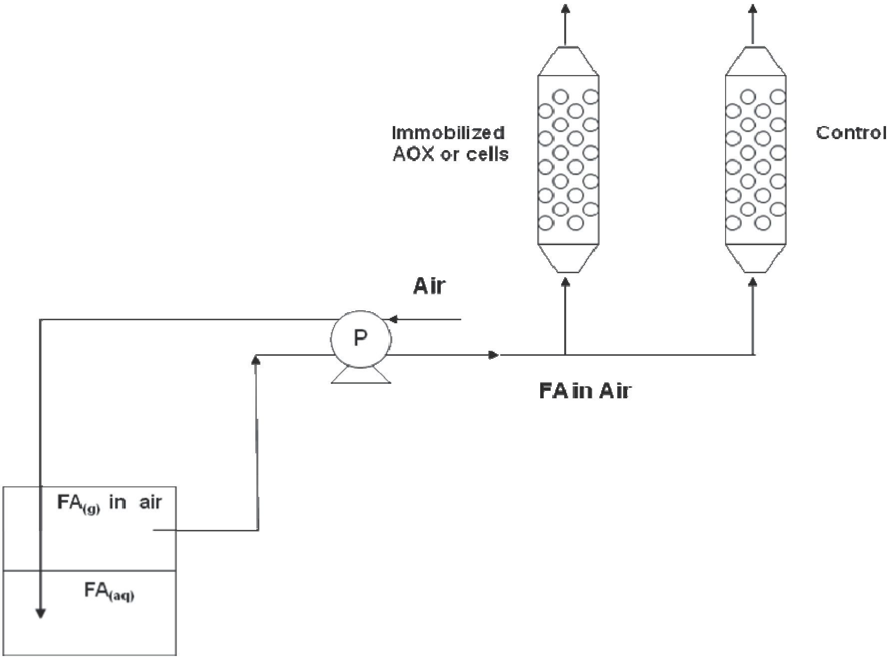


Fig. 15. Scheme for oxidation of airborne FA by AOX immobilized in calcium alginate or cells in a continuous FBBR. 1.5 or 38 g gel beads containing AOX with 6.6 U·g^{-1} of the gel in 20 or 750 ml 0.05 M PBS, pH 7.5, were applied onto a 1x30 cm or 10x10 cm column, which was connected at the bottom to the source of FA in air at 25°C. The 0.3-18.5 ppm FA concentrations in air were generated by bubbling 7-152 ml·min^{-1} airflow through an aqueous FA solution at concentrations of 2.7-100 mM. The control columns contained gel beads without immobilized material. The FA concentrations were tested for about three weeks in the outlet gas phase with a Formaldehyde Gas Detector (Model FP-40 Riken Keiki, Japan) and also in the aqueous column phase by a standard photometric method using a reaction with 1% chromotropic acid (Sawicki et al., 1961), as well as by the amperometric FdDH-based biosensor (Sigawi, 2010).

The proposed method for FA removal from indoor air by the enzyme AOX entrapped in alginate gel provides not only an effective bioconversion of FA in the gas phase, but also a safe FA level in the liquid phase of the continuous FBBR. After termination of the process the contents of the bioreactor can be used as organic fertilizer, since the gel beads together with the liquid phase are free of hazardous components. The entire process can therefore be considered as entirely environmentally friendly. It can be concluded that the proposed bioreactor is suitable for treating air containing various FA concentrations.

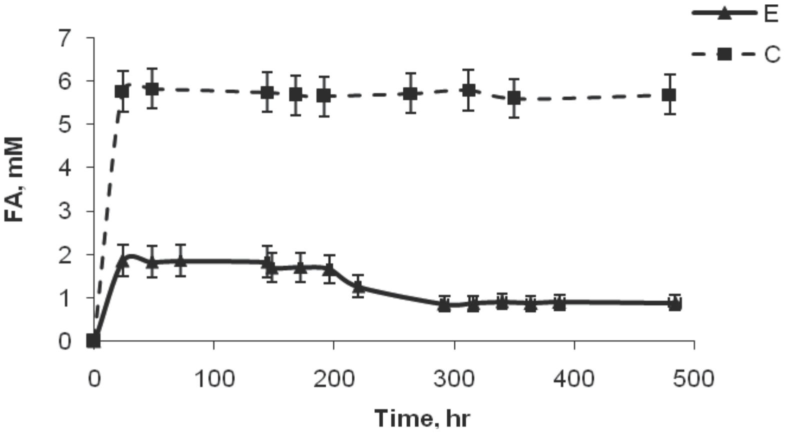


Fig. 16. FA concentration in the aqueous phase of the continuous FBBR upon oxidation of FA in the air by AOX immobilized in 1.5% calcium alginate gel (E). Air flow was 152 ml·min^{-1}, initial FA concentration in air was 18 ppm. The air was bubbled through a 10x10 cm column with 38 g gel beads, containing AOX with 6.6 U·g^{-1} of the gel. FA concentration in the aqueous phase was monitored by a standard photometric method using a reaction with chromotropic acid, as well as by the amperometric FdDH-based biosensor. In the control experiment (C), calcium alginate gel alone was used.

7. Conclusion

Bioremediation of wastes polluted by formaldehyde (FA) and monitoring of this toxic compound in environment, commercial goods, potable water and food products is an important challenge for science and practiclal technology.

In this review, there are described enzymes- and cells-based approaches to monitor FA content in different sources (wastes, indoor air, industrial products, vaccines, and fish food). As the main analytical instrument selective to FA, it has been used recombinant formaldehyde dehydrogenase (FdDH) isolated from the gene-engineered strains of the thermotolerant methylotrophic yeast *Hansenula polymorpha.* The stable recombinant clones, containing 6-8 copies of the target *FLD1* gene, were resistant to 15-20 mM FA in a medium due to over-synthesis of a homologous NAD$^+$- and glutathione-dependent FdDH. A simple scheme for FdDH isolation and purification from the recombinant overproducers was developed, physico-chemical and catalytic properties of the purified enzyme were studied.

The enzymatic method for FA assay, based on recombinant FdDH (with linear detection range from 0.01 to 0.05 mM and detection limit 0.007 mM) and analytical kit "Formatest" were developed. In comparison with the known methods, the described procedure is rather simple: a method does not require transformation of FA into chemical adduct for the extraction of the target analyte from the tested sample. As compared to chemical methods, the analysis time is shorter and some dangerous operations (*e.g.* heating in strong acid) are not required. The developed method is approved on the FA-containing real samples, and data are well correlated with the results of the known chemical methods.

Another FA-oxidizing enzyme, alcohol oxidase (AOX) isolated from the mutant *H. polymorpha* (*gcr1 catX*), defective in glucose repression of AOX synthesis and avoid of catalase, was shown to be useful for enzymatic FA determination in wastes and industrial products. AOX *in vivo* oxidizes methanol, but *in vitro* has ability to catalyze the oxidation of other primary alcohols and hydrated form of FA ($HO-CH_2-OH$). For simultaneous assay of both FA and methanol in wastes, the specific chemico-enzymatic method was elaborated. AOX was also successfully used for FA assay in *Gadoid* fish products.

The purified preparations of FdDH and AOX, as well as *H. polymorpha* cells overproducing these enzymes were used for construction of enzyme-based and microbial electrochemical biosensors selective to FA. The reliability of the developed analytical approaches was tested on real samples of waste waters, pharmaceuticals, and FA-containing industrial products.

AOX and permeabilized mutant yeast cells of *H. polymorpha* (*gcr1 catX*) were shown to be used as the catalytic unit in cartridges for removing of formaldehyde from the indoor air. Experimental data confirm the possibility to exploit the developed bioreactors based on crude preparations of AOX or methylotrophic yeast cells for effective formaldehyde oxidation coupled with FdDH-based biosensor for accurate control of this process.

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7

Satellite Monitoring and Mathematical Modelling of Deep Runoff Turbulent Jets in Coastal Water Areas

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1. Introduction

One of the most important problems at the present time is the environmental pollution. Anthropogenic impacts on seas and oceans, and first of all on coastal water areas where more than half of the Earth population lives, makes the great contribution to this problem.

The most intensive effects on ecosystems of coastal waters have deep waste water outfalls (Israel, Tsiban, 1989). Intensive discharges of pollutants in the near-surface layer of the ocean lead to a progressive eutrophication and microbiological contamination of sea water and cause a disruption in the ecosystem's balance and drop in environmental bio-productivity. Therefore, it is important to organize monitoring of anthropogenic impacts on marine environment caused by deep runoffs.

Study and monitoring of such anthropogenic impacts is usually carried out using in-situ methods, which are of local character. Therefore, the use of aerospace methods and technologies to solve such issues is very promising (Bondur, 2004). This paper gives an overview of contemporary aerospace methods and means, as well as mathematical modelling of turbulent jets caused by deep runoffs, and possibilities of their application to monitor pollutions of coastal water areas. The focus is on the comprehensive studies which allow us to integrate remote and in-situ data with modelling results. For satellite monitoring, the methods are used based on the registration of deformations of surface wave spatial structure and hydrooptical inhomogeneities due to the interaction of deep runoff jets with the ocean surface and the near-surface layer.

Some results from the comprehensive monitoring of anthropogenic impacts on Mamala Bay water area (Oahu Island, Hawaii, USA) are also presented here, as well as data obtained for Black Sea water area near Gelenjik city which confirm these results.

2. Physical features of deep runoff turbulent jet propagation

The dumping of wastewater into the sea is usually from a continually operating source located in the bottom layer. Outfall devices can have various constructions, but all of them, as a result of their operation, create in the marine environment a turbulent jet or a series of jets, density of which differs from water density on the depth of source (Vladimirov et al., 1991). Because the water dumped into the sea is usually non-salty water containing different impurities, its initial density, as a rule, is less than the density of the environment, therefore the jet floats. The main initial parameters of the turbulent jet are the flow rate Q (the amount of liquid moving through the cross-section of the jet in a unit of time), impulse or momentum (the product of the flow rate of a jet and its velocity) and the difference of water density $\Delta\rho$ in the jet (ρ) and in the sea (ρ_0) on the plane of the source, determining together with the flow rate a buoyancy of the jet (Ozmidov, 1986):

$$F = g\Delta\rho Q / \rho_0 , \tag{1}$$

where g is the gravity.

While the turbulent jet propagates, its flow rate increases because of its involvement into the movement of surrounding waters. If the medium is uniform in density and the difference between medium density and a jet density equals zero, when a jet propagates its impulse remains. Otherwise, only the horizontal component of impulse remains whereas the vertical component varies under the influence of buoyancy forces. If the medium is uniform in density, and its density is not equal to the density of the jet, then the jet floats (under the condition of linearity of the equation for state of water).

The main problem of water dumping into littoral water areas is the aim to "bury" jets in under layers, not allowing them to surface and pollute the upper layer. Therefore, outfall devices are installed, as a rule, in the regions with stable stratification (Vladimirov et al., 1991).

If we observe the fluid (Lagrangian) element of the floating jet in stably stratified medium then, because of intermixing with the surrounding waters its density increases, and the density of the surrounding water is reduced (element surfaces) and at the certain depth the difference in densities becomes zero - the jet will cease to surface (Bondur, Grebenyuk, 2001; Zhurbas, 1977).

To facilitate description of jet propagation processes, they are usually divided into 2 phases: active and passive (Bondur, Grebenyuk, 2001).

The active phase is a jet segment from the source up to the place where the level of turbulence (turbulent fluctuations of velocity) in the jet will cease to substantially differ from background, typical marine environment (without a jet). From the similarity principle and dimensions it is possible to establish that in stably stratified medium the duration of the active phase t_0 (time of transition of the fluid element of the jet from the source up to that place where the turbulence in the jet degenerates to levels comparable with the background) is defined as the characteristic time of free oscillations and conditionally can be estimated as a half of Brunt-Väisäla period:

$$t_0 = T / 2 = \pi \left[\left(g / \rho_0 \right) \frac{\partial \rho}{\partial z} \right]^{-\frac{1}{2}} \tag{2}$$

At the end of the active phase, the surfacing of a jet ceases, and its further spreading happens approximately on one plane. Further, the jet makes only damped oscillations relative to that plane on which it was at the end of the active phase.

At the passive phase, the cross-section of a jet becomes significantly non axis-symmetric. Turbulent involvement ceases. Growth in vertical size practically does not happen and even can decrease because of the collapse phenomenon. The horizontal size grows, in particular, due to collapse (Bondur, Grebenyuk, 2001; Ozmidov, 1986). As the jet continues to differ in temperature and salinity from surrounding water on the same plane, on the jet's side borders, Stern thermohaline instability may develop resulting in intrusion and stratification of the jet (Stern, 1960). Thus the jet forms a peculiar vertical "layered cake" and no longer exists as a whole. Intrusion stratification aides growth in the horizontal size of the area occupied by diluted wastewater. As the jet still has a thermohaline anomaly compensated in the density field, on its upper and lower boundaries, convective double diffusion processes like "salt fingers " (on that border where the temperature and salinity decrease with depth) and level-by-level convection (where the temperature and salinity grow with depth) may develop. (Ambartsumyan et al., 1995; Stern, 1960). Convective double diffusion processes can intensify vertical interchange more than on the order, promoting further dilution of dumped waters. Being passive, the jet is transferred by currents, the impurity disperses due to horizontal diffusion. The horizontal size of the area occupied the impurity increases as a result of the combined effects of the vertical shift (gradient) of the currents' velocity and vertical diffusion (so-called diffusion with shift of velocity). Separation of the suspended matter included in the jets occurs. Suspended matter, the density of which is less (more) than the density of water surface (sink) relative to the position of the sewage jet and can reach the surface (or bottom) of the sea. Knowing the hydraulic fineness of the suspended matter, distance from the plane of a surfacing of the jet to the surface (or bottom) of the sea, rate of dilution at the end of the active phase and the effective coefficient of the horizontal diffusion, it is possible to estimate the time of arrival of the suspended matter at the surface (or bottom), the area of contamination and the concentration of the pollutants. The process of separation is not significant in the active phase, because of its short duration (the first tens minutes) (Ozmidov, 1986).

At deep wastewater outfalls lower than the level of the thermocline, there is turbulization of the lower boundary of the density shift, formation of vortex structures of various scales, generation of internal waves, and related fields of currents, shaping of convective motions stipulated by desalting of the under layer of the medium (salt fingers), and appearing of pollutants which cause changes in waver characteristics and affect the water area ecosystem (Bondur, 2004; Bondur, Grebenyuk, 2001; Bondur et al., 2009a).

3. Applicability of space methods for monitoring anthropogenic impacts on coastal water areas

Significant success has been achieved recently in the area of development and application of aerospace methods and technologies for ocean remote sensing. Their main advantages are: wide coverage; real time monitoring; capability to work in any areas of seas or oceans difficult to access; capability to acquire data with various spatial and temporal resolutions and over wide areas of the electromagnetic wave spectrum; measurement of a wide spectrum of parameters; high accuracy of obtained data, especially when combined with in situ measurements; capability to network aerospace information, as well as retransmit data received from monitoring by airplane, helicopter, ships and buoy stations to customers (Bondur, 2004).

Turbulent jets of deep runoffs cause changes in various parameters of the marine environment which can be remotely sensed using aerospace means (Bondur, 2004; Bondur, Grebenyuk, 2001). Deep plume propagation cause deformation of the surface due at direct interaction with surfacing jets, and also generation of surface waves by vortex structures. Besides this, there is a distortion of the space-time structure of the disturbance by internal waves, turbulence and layers of surfactants (Bondur, 2004; Bondur, Grebenyuk, 2001). For registration of such distortions, optical and radar equipment installed on aerospace platforms can be used (Bondur, 2004).

Under the influence of deep plumes, there is also a change hydrooptical characteristic of the seawater, which is exhibited in increased turbidity and changed colour due to the increase of scattering and light absorption owing to a raise in concentration of suspended and dissolved organic matter. To detect such phenomena, it is possible to use passive (spectral, multispectral, and hyperspectral monitoring), as well as active (lidar) optical methods (Bondur, 2004; Bondur, Zubkov, 2005; Bondur et al., 2006a).

Deep plumes cause water surface temperature variations in current fields, upwelling areas, turbulence, and internal waves, etc. For registering changes in temperature, infrared and passive microwave sensing methods (Bondur, 2004; Bondur, Grebenyuk, 2001) can be used.

In the field of the deep plumes there is a change in the physical and chemical characteristics of the marine environment, which is exhibited in magnification of the content of dissolved organic matter, phosphorus, nitrogen, heavy metals, dissolved oxygen, and also in a change of temperature and salinity of seawater. These effects also can be identified using various remote methods. For example, the content of dissolved organic matter can be measured using lidars by analysing spectra of combinational scattering and fluorescence, excited by laser radiation. For measuring the remaining physical and chemical parameters of the environment, the laser-spark method, a method for laser correlation spectroscopy etc. can be used (Bondur, 2004, Bondur, Zubkov, 2001).

Due to the long-term existence of various processes related with deep runoffs, a rise in phytoplankton concentration is possible, resulting in a change in spectral characteristics of the fluorescent signals which also can be remotely registered (Bondur, 2004).

This paper focuses on studying spatial distribution of low-contrast small-scale structures related to deep runoffs. These structures manifest themselves in the changes of surface waves and in heterogeneities of ocean surface layer hydrodynamical and hydrooptical parameters. For this study we use the methods of spatial spectral analysis and the methods of processing high resolution multispectral satellite imagery, as well as the integration of satellite imagery processing results with hydrophysical data obtained during sea truth measurements, as well as with mathematical modelling results.

4. Methodological features of comprehensive satellite and in-situ monitoring of deep runoff impacts on coastal water areas

Comprehensive monitoring of anthropogenic impacts on coastal water areas was performed under the international project. The main goal of this project was detection of negative impact of deep wastewater runoffs on the Mamala Bay water area and Oahu Island resorts ecosystems (Honolulu, Hawaii, USA) (Bondur, 2006; Bondur & Filatov, 2003; Keeler et al., 2003; Gibson et al., 2006; Bondur et al., 2007; Bondur & Tsidilina, 2006).

The main source of anthropogenic load was the discharge of treated wastewaters through the Sand Island outfall device. Its diffuser is 1036 m long and has 282 ports. It is located on

70 m depth and at a distance of 3.8 km from the shore. The average flow rate is 4.48 m^3/s (Fischer et al., 1979).

Fig. 1 presents the scheme of comprehensive monitoring which illustrates the peculiarities of its techniques. In this figure we can see the location of the Sand Island Outfall, images of main satellites, ships, some sensors and remote platforms used for the monitoring (Bondur, 2006).

Periodical space imaging of the bay water area was performed during the monitoring using:

- Optical high resolution sensor of IKONOS and QuickBird satellites providing imagery of 0.61 – 1.0 m resolution and multispectral imagery of 2.44-4.0 m resolution;
- Radar sensor of RADARSAT (8 and 25 m resolution, λ=5.6 cm wavelength, HH polarization) and ENVISAT (~ 25 m resolution, λ=5.6 cm, HH, VV, VH polarization);
- Hyperion hyperspectral sensor (~ 30 m resolution, 220 spectral bands in the range of 0.4-2.5 µm), as well as ALI sensor (~ 10 m resolution (panchromatic mode) and ~ 30 m (multispectral mode), 10 spectral bands in the range of 0.4-2.4 µm of EO-1 satellite;
- ASTER sensor (15 m resolution in visible and near-IR band, 30 m in mid-IR and 90 m in far-IR band), TERRA satellite;
- Multispectral MODIS sensor (250 m resolution in visible and near-IR band, 500 m in mid-IR and 1 km in far-IR band), TERRA and AQUA satellites;
- Multispectral sensors of International Space Station were used (~ 2 m resolution (panchromatic mode) and ~ 5 m (multispectral mode);
- Data acquired by "Meteor-3M", NOAA, and GOES weather satellites were involved.

To verify remote sensing data obtained with space assets, the following sea truth data were used: wind conditions; parameters of surface waves (wave buoys); current fields (ADP sensors and drifters); temperature fields (thermistor strings); temperature and salinity profiles (TS, CTD, and XBT sensors); microstructure data (MSS, TOMI), hydrooptical (AC-9, Secchi disks) and hydrobiological (Niskin bottles) parameters; and tidal mode (Bondur, 2006; Bondur et al., 2007.

Fig 1 shows the locations of stations for measurements of temperature vertical profile time dependences (thermistors), as well as parameters of current velocity fields (ADP). Navigation paths for CTD measurements, determination of hydro-optical (AC-9 sensor and Secchi disks) and hydro-biological parameters, as well as wave buoy paths for surface wave spectra measuring are given in Fig. 1. Dashed line denotes the bay area where vertical and horizontal measurements using dropped and towed microstructure sonde were carried out (Bondur, 2006; Bondur et al., 2007).

Descriptions of sensors used for sea truth measurements are given in (Bondur et al., 2007; Bondur & Filatov, 2003; Gibson et al., 2006; Keeler et al., 2004; Wolk et al., 2004).

A large number of satellite images from various satellites were obtained during experiments carried out in the Mamala Bay water area. The total data volume exceeded 150 Gb (Bondur, Tsidilina, 2006).

Original satellite images and sea truth data, as well as complementary information (digital maps, meteorological and GPS data, etc.) were recorded to the archive. Then, data pre-processing was carried out to enhance image quality. After pre-processing, the data was subjected to further thematic processing and comprehensive analysis (Bondur & Tsidilina, 2006).

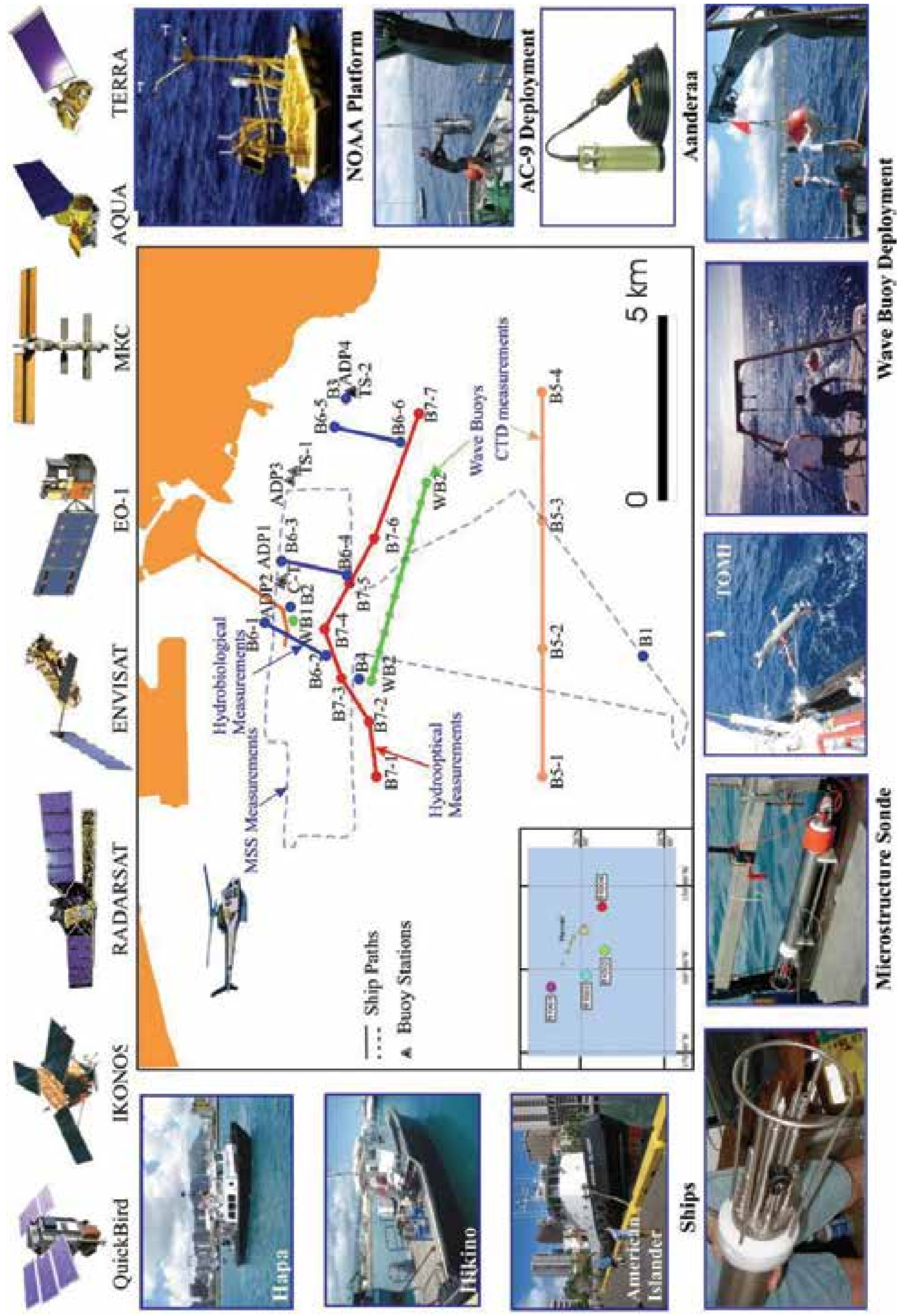


Fig. 1. Schematic for comprehensive monitoring of the coastal water area near Oahu Island (Hawaii)

5. Comprehensive monitoring results

5.1 Detection of “quasi-monochromatic” structures in the areas of deep outfalls using satellite imagery

Among the most important monitoring results was the detection in high resolution satellite imagery of “quasi-monochromatic” structures in the area where disturbances caused by deep plumes interacted with surface waves.

The processing of high resolution satellite images was carried out using a technique based on the method of remote spatial-frequency spectrometry (Bondur, 2004). The original satellite images were divided into fragments of 1024x1024 and 2048x2048 pixels, ensuring sampling volumes sufficient to attain required statistical precision for spatial spectra evaluation and achieve the spatial resolution needed to estimate geometrical characteristics of surface anomalies caused by deep outfalls. These fragments were used to evaluate 2D-spatial spectra and their cross-sections in different directions; to determine informative indicators of these spectra; to compute parameters of spectral harmonics; to carry out statistical analysis of informative indicators; to detect abnormal areas related to the deep outfall and to evaluate dimensions of these anomalous areas. The technique described above was used for the processing of IKONOS and QuickBird imagery obtained for the days of comprehensive experiments in the Mamala Bay water area (Hawaii, USA) and in Gelenjik Bay (Russia).

Fig. 2 shows an example of the processing of the IKONOS satellite image taken on September 2, 2002. The satellite image fragment in Fig. 2,a shows three areas highlighted to the south from the outfall diffuser (orange colour) in the area of estimated plume manifestation (anomaly) and one area far from the diffuser (background). Fig. 2,b shows 2D spatial spectrum of the background fragment, and Fig. 2,c shows three spectra of anomalous fragments in the outfall area (conditional colours). As can be seen in Fig. 2a,c, the spectra of the anomalous fragments clearly show additional narrow “quasi-coherent” spectral components (spatial frequency $\nu \sim 0.01075\ m^{-1}$, average spatial period for these components is $\overline{\Lambda} = 93$ m, and average widening is $\Delta\overline{\Lambda} \sim 4$ m). Thus, the condition

$$\Delta\Lambda << \Lambda\,, \tag{3}$$

is fulfilled, therefore such additional wave components can be called “quasi-monochromatic”.

Their generation is related with surface manifestations of disturbances caused by turbulent jets of deep outfall. There are no such spectral harmonics in the background spectrum (Fig. 2,b).

Fig. 2,d,e shows one dimensional frequency spectra obtained using wave buoys at the moment of space imaging (11 hours, 20 minutes, local time) in the outfall area (e) and in the background area (d). As can be seen from the one-dimension spectrum in Fig. 2,e an additional “quasi-coherent” spectral component corresponds to spatial frequency $\nu=0.01075\ m^{-1}$ (wavelength $\Lambda \sim 93$ m), what conforms to the spatial periods of “quasi-monochromatic” components detected by 2D spectra of the space image fragment showing the anomaly.

Fig. 2,f shows the result from identification of a surface anomaly caused by the deep outfall. This anomaly was estimated taking into account changes in spatial spectra of high resolution satellite imagery caused by additional “quasi-coherent” spectral harmonics.

In this figure, anomalous fragments of the image (1x1 km^2 size) which correspond to two dimensional spectra displaying “quasi-monochromatic” wave components are filled with

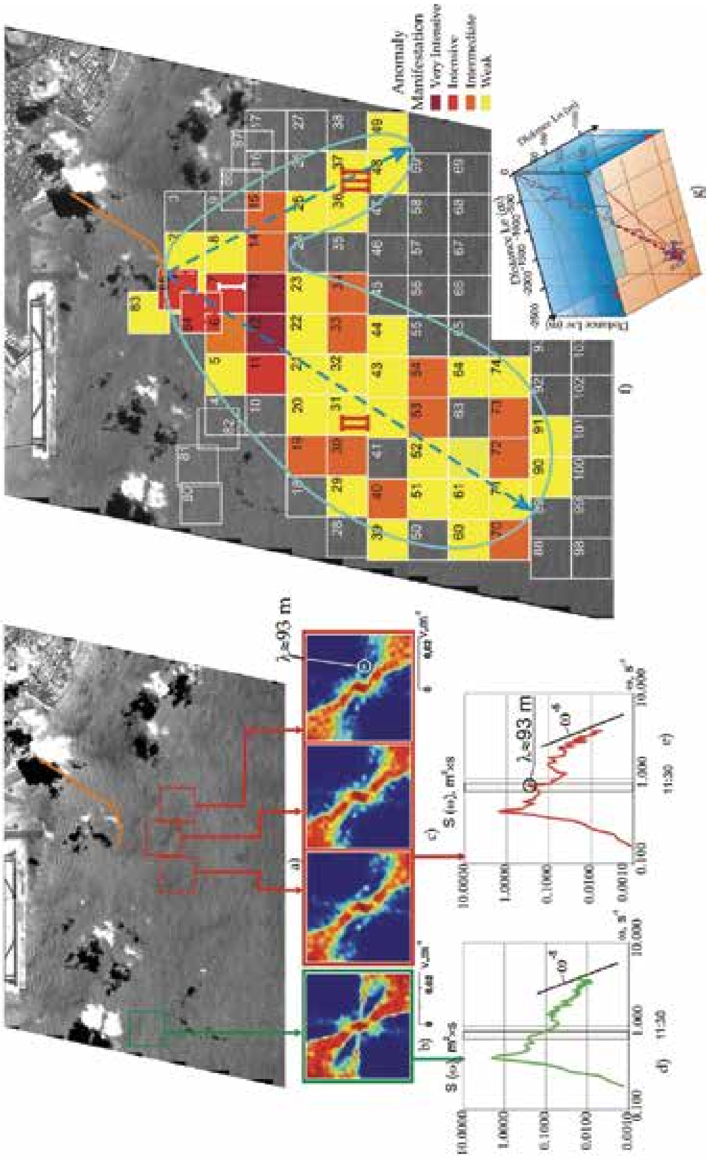


Fig. 2. Revealing surface anomaly using satellite image (IKONOS, ~ 1 m resolution, 2 Sept. 2002). This anomaly is caused by deep outfall impact on surface waves, and is detected by the generation of “quasi-monochromatic” components. a - fragment of the initial image with 4 pieces for processing (in the deep outfall area (anomaly) and background); b - 2D background spectrum; c - 2D spectra of anomalous fragments with “quasi-coherent” spectral harmonics with Λ=93 m wavelength; d – one-dimensional frequency spectra obtained by wave buoys in the background area; e - one-dimensional frequency spectra of deep outfall area (with “quasi-coherent” spectral harmonic corresponding to 93 m wavelength); f - satellite image with detected during the processing anomalous areas of various intensity (very intensive, intensive, medium, weak) related with “quasi-monochromatic” wave component s; g - progressive-vector diagram for current velocity field

different colours. The colours correspond to various anomaly intensities, depending on the intensity (energy) of additional harmonics. The entire zone of anomalies zone (outlined in blue) has the two-lobe "mitten-like" shape, the larger lobe of which (length more than 11 km and width of about 6 km) is elongated in the south west direction (angle about 215°) and the second, smaller lobe (length about 6-7 km, width about 2 km) is stretched in the south east direction (with angle about 154°).

Fig. 2,g presents a progressive-vector diagram based on current velocity data for the experimental area (Bondur & Filatov, 2003). The analysis of Figs. 2,g and 2,f shows that the dominant direction of the elongation of the surface anomaly with "quasi-monochromatic" wave components almost coincide with dominant current direction at the moment of the satellite imaging.

Some example results of surface anomaly detection are given in Figs. 3 and 4. These anomalies are related with deep outfalls and appear as additional "quasi-monochromatic" wave components.

Fig. 3 shows the results of spatial spectral processing of the QuickBird space image obtained on September 14, 2003.

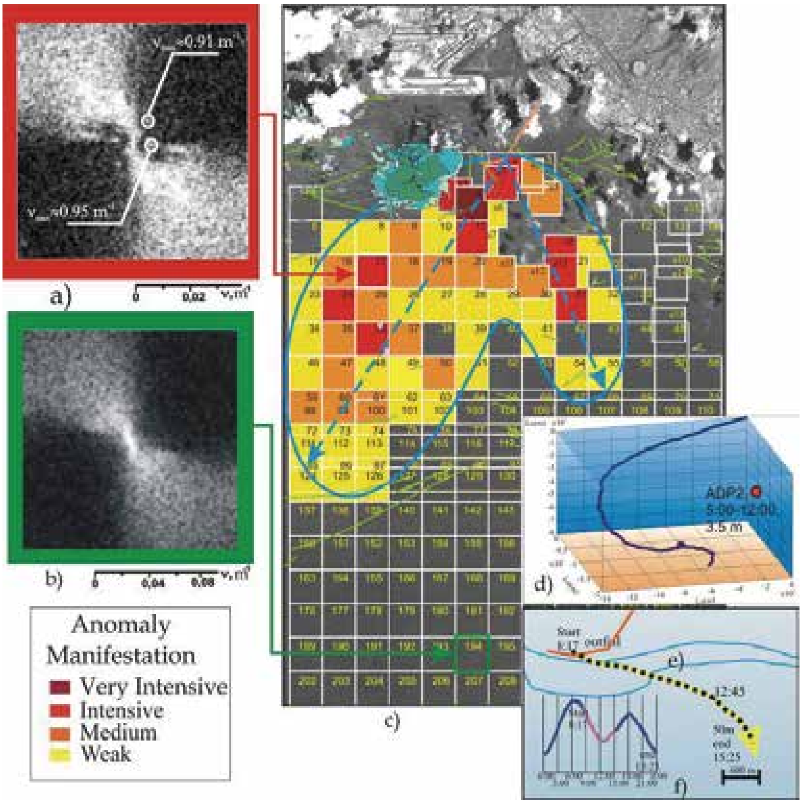


Fig. 3. QuickBird image processing results (September 14, 2003). a) – 2D spatial spectrum with two pairs of "quasi-coherent" spectral maxima in the anomalous area; b) – Background spectrum; c) – The anomaly due to "quasi-monochromatic" wave components in the area of deep outfall; d) – Current field progressive-vector diagram (ADP measurements from 5:00 till 12:00 LT at a depth of 3.5 m); e) – Drifter moving trajectory; f) – Tide diagram

As for the experiment whose results are shown in Fig. 2, on the September 14, 2003 near the diffuser, additional "quasi-coherent" spectral harmonics were detected (Fig. 3,a) and were not detected (Fig. 3,b) far from the diffuser (background). However, as opposed to the case from Fig. 2, in this experiments there were detected two pairs of additional narrow spectral harmonics (see Fig. 3,a)with wavelengths Λ_1 = 91 m and Λ_2 = 95 m matching the condition (3). And the pairs of these "quasi-coherent" spectral harmonics were oriented in different directions, what indicates generation of two systems of "quasi-monochromatic" wave components on the sea surface propagating in different directions (Fig. 3,a).

Even more complicated wave pattern was detected during the processing of images taken on other days. E.g., a great number of pairs of narrow spectral maxima differently oriented were revealed in this QuickBird image (September 3, 2004) fragments (1.33 × 1.33 km^2) (see Fig. 4,b). These maxima matched the condition (3) what indicated the presence of a multimode system of "quasi-monochromatic" wave components on the sea surface (see Fig. 4). Average wavelengths were 43 to 117 m (see Fig. 4,b). There are no such "quasi-coherent" harmonics in background spectra (Fig. 4,c).

Unfortunately, the lower right part of satellite imaging area was covered with clouds, what did not allow us to study the anomalies in this area in more details.

Shapes of surface anomalies detected in the deep outfall area on September 14, 2003 (See Figs. 3,c) and September 3, 2004 (See Fig. 4,a) taking into account spectral harmonics are similar (mitten-like shape), though have some differences (size, direction, intensity, inner structure).

The processing results for satellite images obtained on different days of experiments indicate the repetition of observed surface interests related with deep outfall impacts on Mamala Bay water area. A similar stable form may result from the regular south-western transfer of water mass in the surface layer (~220^o - 235^o) detected in data collected using the ADP (Acoustic Doppler Profiler) (Bondur & Filatov, 2003; Bondur et al., 2007). This can be seen in Fig. 3,d which presents a 3D progressive vector diagram built based on data collected by the ADP2 station (the closest to the diffuser) at a depth of 3.5 m on September 14, 2003. The analysis of current fields enables us to conclude that the south-western direction of water mass transfer prevailed at all depths for a long time (currents velocity was in the range of 10-20 cm/s). However, this direction has changed to the southeast near the time of space imaging (September 14, 2003, 11:16 LT). This is related with the change in tidal phase (see Fig. 3,f). This resulted in the appearance of two lobes in the surface anomaly detected by space imagery. South-eastern propagation of wastewaters was also revealed in current measurements carried out using a Lagrange drifter near the diffuser (see Fig. 3,e) (at a depth of about 50 m, velocity 13 cm/s) (Bondur et al., 2007).

These results are similar to those obtained on other days of experiments.

Summarising all obtained data for Mamala Bay, let's indicate that average wavelengths of "quasi-monochromatic" waves varied between $\overline{\Lambda}$ = 30…200 m over different points of surface anomaly and different days. And directions of anomalous spectral harmonics varied between 45° and 180°.

The shape of surface anomaly is usually two-lobe (mitten-like) with max size of 11 to 25 km.

5.2 Physical mechanisms causing "quasi-monochromatic" wave components in deep outfall areas

Appearance of surface wave anomalies related to discharges of non-salty water into salty marine environment is caused by a series of hydrophysical mechanisms (turbulence,

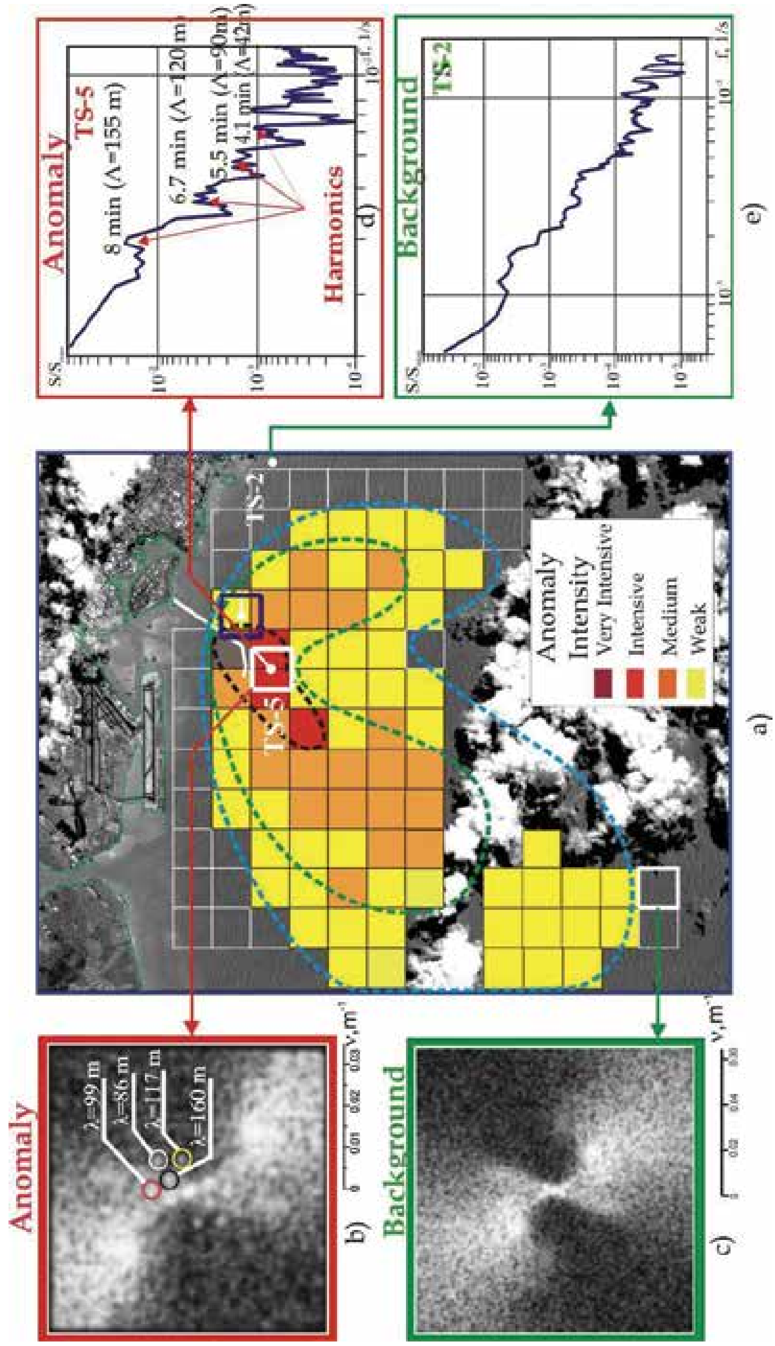


Fig. 4. Detection of "quasi-monochromatic" wave components in satellite image (QuickBird, September 3, 2004) spectra: original image showing deep outfall manifestation area - (a); spectrum with multimode "quasi-coherent" harmonic system - (b); background spectrum - (c); internal wave spectra near the diffuser (TS-5) - (d); and outside the anomaly (TS-2) with indicated periods and appropriate lengths of internal waves - (e)

buoyant vortexes, internal waves, oil and surfactant films, and etc.), most important of them is an interaction of short-period internal waves and surface waves (Bondur, 2004; Bondur, Grebenyuk, 2001). To confirm this, let's analyze spectral parameters of internal waves near and at the distance from the diffuser. The results of measurement of temperature profiles (every 30 s) using moored Thermistor Strings are used for this purpose. These results are to be compared with characteristics of "quasi-monochromatic" wave components on the sea surface detected taking into account spatial spectra of satellite image in the area of deep outfall.

The comparison will be made for the case of experiments in Mamala Bay on September 3, 2004. The results of fragment-by-fragment (1.33x1.33 km^2) spatial spectral analysis of QuickBird satellite image taken on that day are shown in Fig. 4,a. As we see in Fig. 4,b, multimode structure of narrow "quasi-coherent" spectral harmonics are clearly seen in the anomalous spectrum. These harmonics are absent in the spectrum for background (Fig. 4,c). These spectral harmonics correspond to the surface wave systems with average lengths $\overline{\Lambda}$ = 43; 86; 99; and 117 m. Average width of these harmonics is $\overline{\Lambda}$ ~ 5 – 7 m. Thus, the condition $\overline{\Delta\Lambda} << \overline{\Lambda}$ is fulfilled that allow us to term these detected components "quasi-monochromatic".

Study of high-frequency internal waves will be carried out using analysis of 27.5°C isotherm depth spectra for 2 hour time period (10:00 – 12:00 LT). (Bondur & Filatov, 2003; Bondur et al., 2007).

Fig. 4,d presents an internal wave spectrum created using data obtained at TS-5 located 600 m to the south from the outfall diffuser (see Fig. 4,a). A similar spectrum for TS-2 far from the diffuser near Waikiki Beach (see Fig. 1) is given in Fig. 4,e). Unlike in the background spectrum (TS-2), ultrahigh frequency spectral components (with 4.1 – 8 min periods) caused by internal waves generated by deep outfall are clearly seen in the spectrum of T=27.5°C isotherm depth near the diffuser.

Lengths of such ultra short internal waves estimated based on measurement results for their periods using the dispersion relation for such waves and measured in the experiment Brunt-Väisälä frequencies $N=(gd\rho/\rho dz)^{1/2}$, where ρ is the density; z is the depth.

The internal wave period is $T_{IW} = 2\pi/\varpi_{IW}$.

The internal wave frequency is derived from the dispersion relation:

$$w_{IW} = gk_{IW}\frac{\Delta\rho}{\rho}\left[cth(kh)+cth(k_{IW}H)\right]^{-1} \tag{4}$$

where:

$k_{IW}= 2\pi/\Lambda_{IW}$ - is the wave number for internal waves;

Λ_{IW} is the internal wave length;

$\Delta\rho = \rho_2 - \rho_1$ is the difference between lower and upper layer density.

The phase velocity of internal waves is the following:

$$C_{IW} = \frac{w_{IW}}{k_{IW}} \pm \left\{g\frac{\Delta\rho}{\rho}/k_{IW}\left[cth(k_{IW}h)+cth(k_{IW}H)\right]\right\}^{1/2} \tag{5}$$

In our case we deal with ultrashort internal waves matching the following condition:

$$k_{IW}h >> 1 \text{ and } k_{IW}H >> 1 \tag{6}$$

Taking into account (5) and (6), the phase velocity is the following:

$$C_{IW} = \left(\frac{\rho \Delta \rho}{\rho} \right) / \, 2k_{IW} \tag{7}$$

The internal wave length is:

$$\Lambda_{\mathrm{IW}} = T_{\mathrm{IW}} \cdot C_{\mathrm{IW}} \tag{8}$$

Using these equations and taking into account the measurement results, wavelengths for our case are Λ = 42; 90; 120 and 155 m for appropriate spectral maxima (see Fig. 4,d). Similar harmonics are absent in the internal wave background spectrum (see Fig. 4,e), what is the evidence of their direct relation with such a determined source as a permanent deep outfall. The comparison of lengths of ultrashort internal waves (TS-5 Station) with "quasi-monochromatic" harmonic lengths of 2D spatial spectrum for the satellite image fragment covering the area around this station confirms their very good correlation (1 – 5 % difference). This is the evidence of the validity of "internal wave" hypothesis of generation of multimode system of "quasi-monochromatic" surface structure caused by a deep outfall.

Physical mechanisms of internal wave interaction with surface waves were analysed in (Bondur, 2004; Bondur, Grebenyuk, 2001).

5.3 Radar satellite imagery analysis

Radar imaging using the RADARSAT and ENVISAT satellites was carried out during the monitoring of anthropogenic influence caused by the deep outfall in the Mamala Bay water area. Radar image pre-processing consists of calibration (σ^0 (sigma nought) calculation), speckle-noise elimination, geo-coding and formation of improved images by applying equalization procedures. Thematic processing included the following main stages: scanning of pre-processed imagery by a statistically significant sliding window, and calculation of informative attributes in each window, as well as statistical analysis, choice of optimal informative attributes and automated classification (Bondur, 2004; Bondur, Starchenkov, 2001).

To verify the results of radar image processing, data on wind fields obtained by ships and ground stations, as well as hydrophysical data were used. Use of additional information allowed us to decrease error probability during classification of radar imagery down to 0.04-0.06 using the Neyman-Pearson criterion.

Detection of areas related to the deep outfall and anomalies caused by the dynamic influence of wind was carried out using automated classification procedures based on various types of informative attributes (Bondur, 2004; Bondur, Starchenkov, 2001). Physical mechanisms of ocean surface radar image formation were described in (Bondur, 2004), as well as registered parameters were analyzed. The presence of disturbed ocean surface revealing in the variations of Bragg component of reflected radio waves (Bondur, 2004) made possible the detection of the deep outfall in Mamala Bay water area against the anomalies caused by wind flows from Nuuanu Valley and Manoa Valley.

The results of anomaly detection induced by Sand Island Outfall are given in Fig. 5. In this Figure different colours designate various radar signal intensities. Such surface anomalies are caused by deformations of high frequency (5 – 10 cm wavelengths) surface waves under the influence of deep outfalls which can be registered by a radar (due to Bragg mechanism) (Bondur, 2004).

Maximal dimensions of the surface anomaly caused by wastewater discharge from Sand Island are: max length is 24 km, max width is 7.6 km. The direction of elongation is ~250°, what matches the dominant current directions at the moment of radar imaging.

Max length of the anomaly due to Honouliuli outfall is ~5 km, and max width is ~2.3 km.

More details of the results of radar imagery obtained during monitoring can be found in (Bondur, 2004; 2005; Bondur, Starchenkov, 2005).

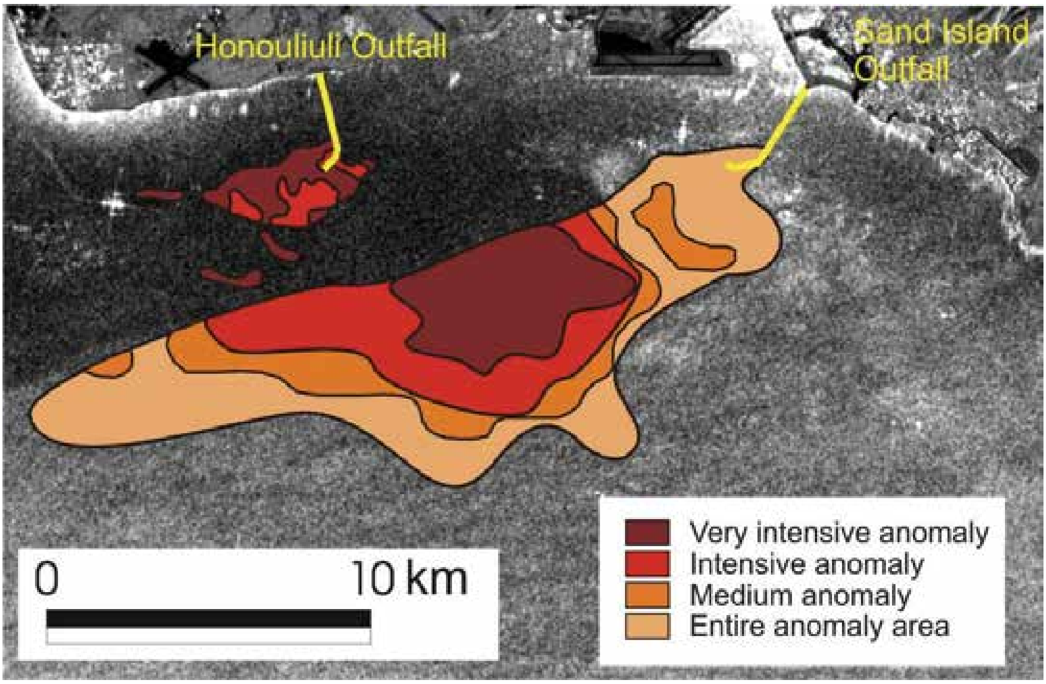
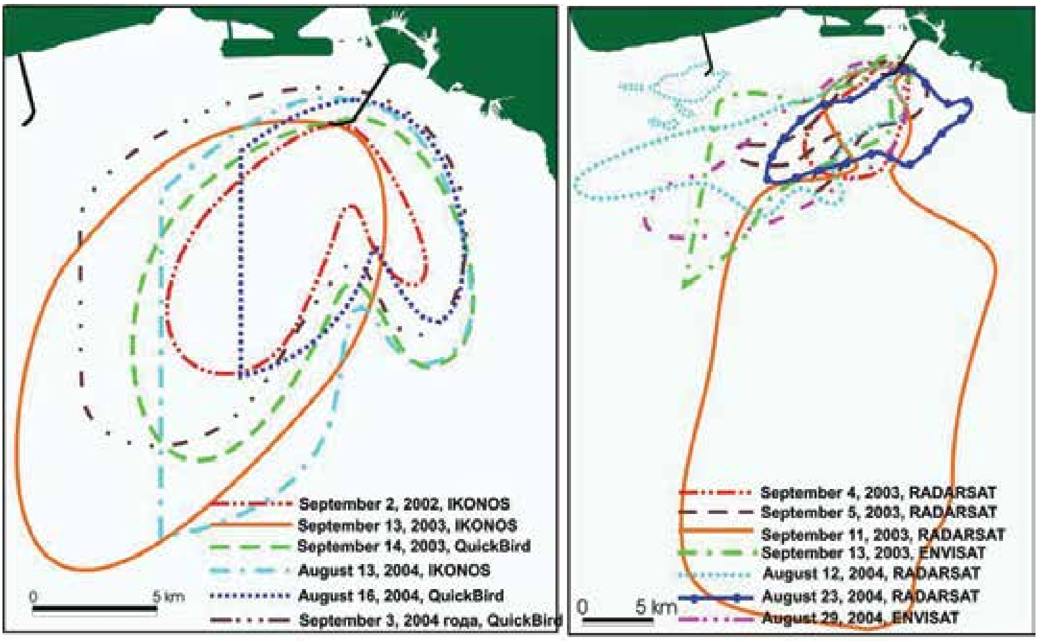


Fig. 5. Detecting the anomaly caused by the Sand Island and Honouliuli outfalls using the RADARSAT image of August 12, 2004

5.4 Summarizing surface effects of anomalies caused by deep outfall and detected using satellite imagery

Contours of anomaly propagation areas caused by a deep outfall and detected by spatial spectral processing of optical satellite imagery (a) and radar satellite imagery (b) are summarized in Fig. 6. These results were obtained for various days of comprehensive monitoring under various meteorological conditions (tide-and-ebb phases, current and wind directions and velocities, surface waves, thermocline location, etc.). The analysis of Fig. 4 shows that despite having significant difference (in dimensions, shape, propagation area) in the studied anthropogenic influence manifestations they have definite common features for each of data class obtained using various sensors. These features are appearing in rather local nature of similar anomaly manifestations, and relative shape stability for each type of sensors. The exclusion is the anomaly detected from RADARSAT image (September 11, 2003) under conditions of calm (wind speed was 1-1.5 m/s) (Bondur, 2004; 2005).

Differences in anomaly manifestations detected from optical and radar imagery denote various significant environment parameters registered by these sensor types.



a) b)

Fig. 6. Propagation areas for anomalies caused by the deep outfall in Mamala Bay (Hawaii)detected in optical (a) and radar (b) satellite images for different days under various hydrometeorological conditions

5.5 Anomalies of hydrooptical characteristics detected using high resolution satellite imagery and sea truth data

The processing of high resolution (2…4 m) multispectral images was carried out using the characteristics of relative signal variety in red (R), green (G), and blue (B) spectral bands of 60 – 80 nm width. The processing technique used the following basic procedures (Bondur, 2004; Bondur, Zubkov, 2005): synthesizing the colour image from separate bands (RGB-synthesis); interpreting imagery to mark out clouds, ships and their traces, land, and unclouded marine surface; selecting fragments of the full scene of an image for the area of interest for further processing; filtering; decorrelation stretch to remove correlation of spectral bands; parametric and non-parametric classification; combination of classes; colour coding.

To correct brightness image distortions caused non-uniform sensitivity of the CCD camera, additional procedures consisting in removing brightness transversal trend within each fragment; and brightness band interleveling based on statistic parameter use.

To verify the results of multispectral satellite imagery processing in the studied area, sea truth measurements were carried out using AC-9 hydrooptical equipment and various hydrophysical equipment at the moments of time close to satellite imaging time (Gibson et al., 2006; Bondur et al., 2006a; 2007)/ The gauge was deployed from the *Klaus Wyrtki* ship down to a depth of 150 m. Values of absorption factor and attenuation were measured using AC-9 equipment at nine wavelengths (in 412 to 715 nm spectral band) at each station (B6) located in the area of the outfall. Vertical profiles of these values were created for each station (Bondur et al., 2006a). To process AC-9 data we used the method based on the Haltrin-Kopelevich linear bio optical model (Kopelevich, 1983; Haltrin & Kattawar, 1993).

Fig. 7 presents the examples of multispectral QuickBird image processing (September 14, 2003; 11:16 LT imaging time). In this Fig. we can see: image fragment (16.5 x 16.5 km^2) synthesized from RGB bands of the original image (a); interim processing result consisting in obtaining pixel-by-pixel band signal ratios blue/green, in a convolution with mask and classification with further smoothing (b); result of combination of classes of similar brightness with colour palette changing (c); re-combination of classes, detection and outlining of anomalies (d).

The analysis of processing result shows that in the area of the Sand Island outfall diffuser (right part of Fig. 7,d) anomaly of subsurface ocean layer hydro-optical characteristics is evident.

Maximal size of this anomaly is about 6 km. Inside of this area more contrast extensive anomaly (~ 3.5 km length) oriented in south direction, is detected. Another distinct surface anomaly caused by oil spill due to leakage from a tanker during pumping to onshore reservoirs is evident. Rather small anomaly of hydro-optical characteristics caused by another outfall (Honouliuli) in Mamala Bay is seen on the left (see Fig. 7,d). Effectiveness of the applied processing technology is confirmed by the fact that on original images anomalies caused by the outfall are not seen.

Similar results were obtained after processing other multispectral satellite imagery as well as multispectral data (Bondur, 2004; Bondur, Zubkov, 2005; Bondur et al., 2006a).

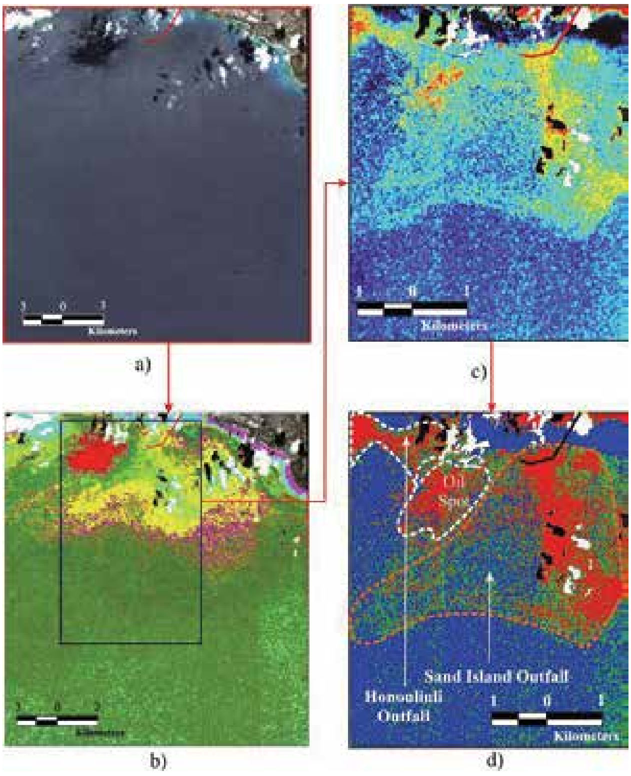


Fig. 7. Example of QuickBird multispectral image processing. a) original synthesized images; b) processed fragment; c) classification with smoothing by a window; d) combination of classes; e) final result

For the comparison with satellite imagery processing results, absorption and attenuation factors were used which had been obtained from AC-9 data at the wavelength of λ=0.488 µm, where sunlight absorption near the Hawaii was close to the minimum (Erlov, 1980). Also, AC-9 spectral band coincided with the centre of QuickBird blue band.

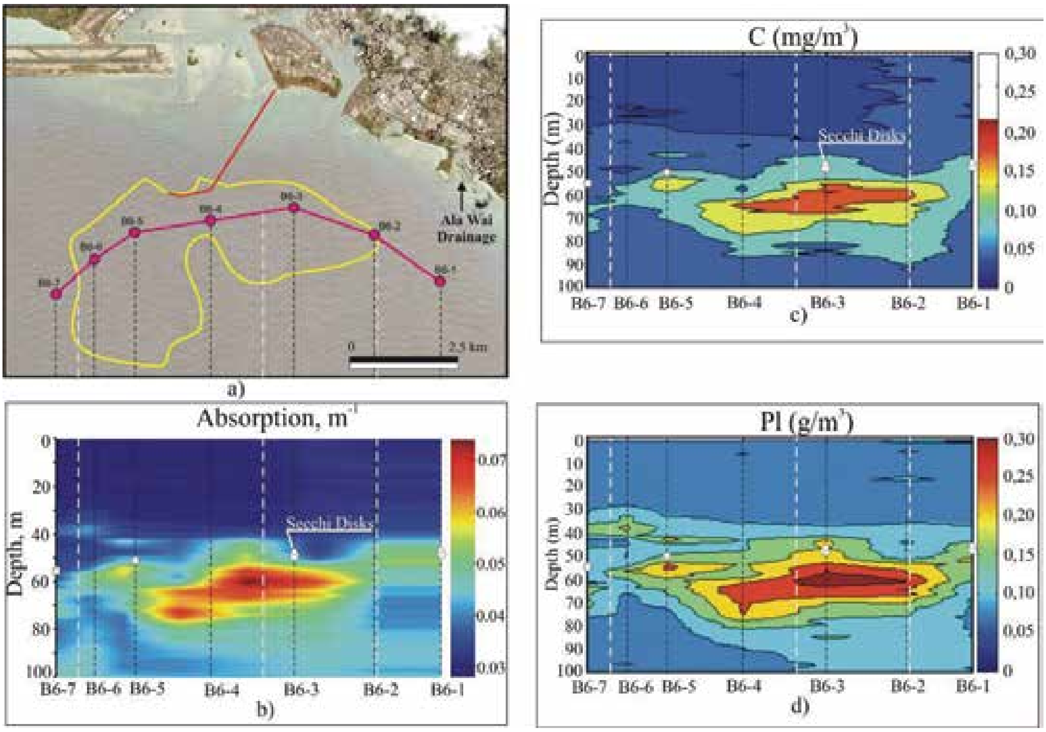


Fig. 8. Comparison of the anomaly detected using QuickBird multispectral imagery (September 3, 2004) (a) with 2D cross-sections of absorption at 0.488 µm wavelength (b); chlorophyll C (c) and large particles (d) concentrations based on AC-9 data. ○ – Secchi disk max visibility (b-d)

Fig. 8.a presents the outlined area of hydrooptical parameter anomaly detected using the multispectral QuickBird image of September 3, 2004 near the deep outfall and ship trajectory with indicated points where hydrooptical measurements had been carried out. Fig. 8 shows 2D distributions of absorption at λ = 0.488 µm (b), as well as chlorophyll C (c) and large particle (d) concentrations based on AC-9 data.

The results obtained by Secchi disks have shown than at B6-3 and B6-5 Stations (near the diffuser) maximum visibility was 48-51 m, while at B6-7 Station (far from the diffuser) it was 55.5 m. It is evident, that at B6-3 and B6-5 Station visibility decreased because of high concentrations of various substances (organic, suspended particles, end etc.) contained in wastewaters.

The processing analysis have shown the high level of coincidence both of western and eastern anomaly boundaries detected using the satellite multispectral images with the anomaly detected using hydrooptical data. The divergence of the results is 100 – 200 m.

Similar results were obtained during multispectral and hyperspectral satellite data (HYPERION). Max anomaly size was 5 – 20 km (Bondur, 2004); Bondur & Zubkov, 2005; Bondur et al., 2006a).

Thus, the comprehensive analysis of the collected data have allowed us to interpret unambiguously the processing results for multispectral imagery obtained during the monitoring of anthropogenic impacts on the water environment.

6. Modelling the propagation of turbulent deep plumes

6.1 The model employed

A mathematical model described in (Bondur, Grebenyuk, 2001; Bondur et al., 2006b; 2009b) has been used to study the propagation features of turbulent jets of contaminated waters discharged into Mamala Bay. The jet propagation is described with a system of seven ordinary differential nonlinear equations that characterize the balance of the horizontal and vertical components of the momentum, the heat consumption, the salinity, and the jet coordinates with the system being supplemented with the equation of the state of the sea water. These equations have been obtained by integrating the equations of the motion, continuity, and heat and salt balance under the assumption of scaling of the distributions of the velocity, temperature and salinity in the cross section of the jet (Bondur et al., 2006b).

When deriving the equations, we considered a turbulent jet that was injected at the depth *z* into the aquatic medium at angle of Θ_0 to the sea line in the *xz* plain. The medium was assumed to be incompressible and quiescent, and its density $\rho_a(z)$ was depth dependent with $d\rho_a/dz < 0$, which means the stable stratification of the medium (Bondur et al., 2006b).

The equation system looked as follows (Bondur et al., 2006b; 2009b):

$$\frac{d}{ds}(ub^2) = 2\alpha ub\,, \tag{9}$$

$$\frac{d}{ds}(u^2b^2\cos\Theta) = 0\,, \tag{10}$$

$$\frac{d}{ds}(u^2b^2\sin\Theta) = 2g\lambda^2b^2\frac{\rho_a-\rho_0}{\rho_0}\,, \tag{11}$$

$$\frac{d}{ds}[ub^2(T_a-T)] = \frac{1+\lambda^2}{\lambda^2}b^2u\frac{dT_a}{ds}\,, \tag{12}$$

$$\frac{d}{ds}[ub^2(S_a-S)] = \frac{1+\lambda^2}{\lambda^2}b^2u\frac{dS_a}{ds}\,, \tag{13}$$

$$\frac{dx}{ds} = \cos\Theta\,,\ \frac{dz}{ds} = \sin\Theta \tag{14}$$

$$\rho = \rho(T,S) \tag{15}$$

where $T_a(s)$ and $S_a(s)$ are the temperature and salinity of the medium, $T(s)$ and $S(s)$ are the temperature and salinity of the jet; $\alpha = 0.057$ is the entrainment coefficient; $b = b(s)$ is the characteristic half-width of the jet, and 1 = 1.16 is a constant; *s* is the coordinate along the jet axis, *r* is the radial coordinate, *u(s)* and ρ(s) are the jet's axial velocity and density, $\rho_0 = \rho_a(0)$ is the reference density.

This system can be supplemented by an equation for the mean time t of the propagation of a fluid element along the trajectory of the jet:

$$dt = \frac{ds}{\overline{u}} = \frac{2ds}{u}, \tag{16}$$

where the mean velocity is determined from the condition that the Gaussian distribution of the velocity is substituted with a constant velocity $\overline{u}$ = u/2 in the section of the jet with a radius $\overline{b} = \sqrt{2b}$ at constant discharge and momentum.

The use of this model (9) – (16) makes possible the calculation of the resulting depth and the thickness of the jet propagation layer (the Ozmidov scale (Ozmidov, 1986)) in the stratified medium, dilution, and other parameters. A detailed description of the model is given in (Bondur et al., 2006b; 2009b).

6.2 Modelling results

When performing the model calculations, the following specifications of the Sand Island facility were used: the mean total discharge rate was Q = 4.64 m^3/s, the mean rate of the discharge from a single diffuser orifice was Q_0 = 0.0163 m^3/s, the velocity of the jet exiting the diffuser orifices was U_0 = 3 m/s, the depth level of the diffuser site was H = 70 m, and the temperature of the discharged waters was T_C = 25-27.5°C (Fischer, 1979). It was supposed that non-salty water discharge took place.

The data of the hydrophysical measurements (Bondur et al., 2007; Bondur & Tsidilina, 2006; Gibson et al., 2006; Wolk et al., 2004) were used to understand the stratification of the aquatic medium. It is worth noting that there are strong tidal currents that substantially influence the diverse hydrophysical processes, including the propagation of the turbulent jets of the discharged waste water (Bondur et al., 2008, Bondur et al., 2006a; Bondur & Filatov, 2005; Merrifield & Alford, 2004).

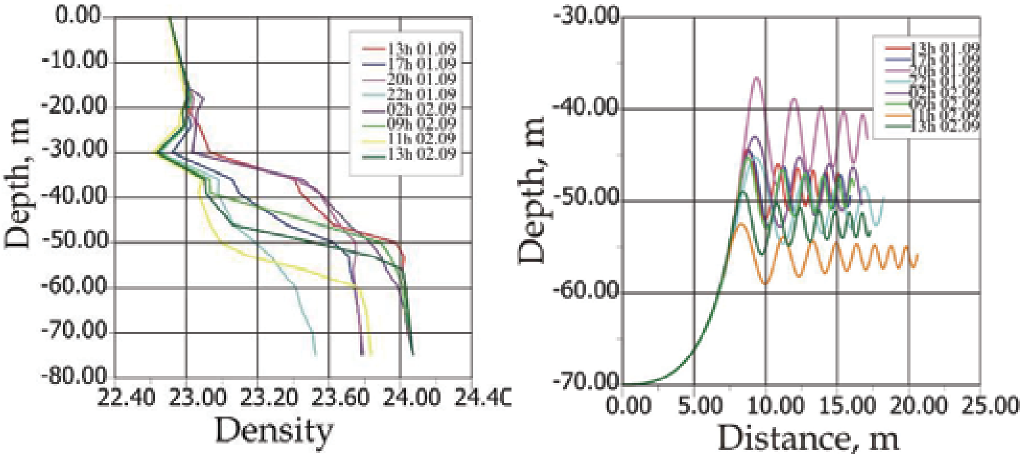
The hourly mean vertical density profiles plotted for eight time moments during the period from 13:00 September 1 to 13:00 September 2, 2002, are shown in Fig. 9,a. During this period of research, the intense density jump layer was located at depths of 30-50 m. The trajectories of propagation of floating-up jets in the mentioned time periods are shown in Fig. 9,b.

The graphs of the level of the floating-up jet and the density gradients for eight time moments during the period from September 1 to September 2, 2002, are shown in Fig. 10,a.

It is seen from these figures that, in the period considered, the jet did not rise higher than 36 m, i.e., not higher than the location of the density jump. The density jump with a strong gradient prevented the floating up of the jet closer to the surface.

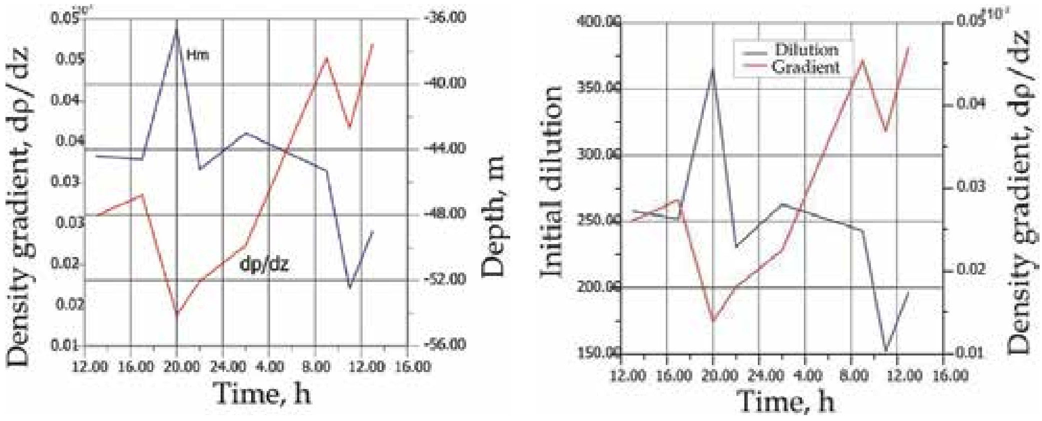
Using the model developed, we also obtained estimates of the initial dilution of the sewage water. The graphs of the variation of the dilution Q/Q_0 and the density gradient $\Delta\rho/\Delta z$ for the period of research are shown in Fig. 10,b. It is seen from this figure that the weakest stratification of the seawater corresponds to the maximal value of the dilution of the discharged waters.

The outcomes of the model calculations of the initial dilution and the jet floating-up depth at thermistor chain locations from August 14 until August 26, 2004 are shown in Figs. 11,a,b. Under the stratification conditions characteristic of the site of station Ta, the jet remained mainly submerged (Fig. 11,b), excluding the shorter time periods when the diffuser occurred at the base of an internal tidal wave of large amplitude, when the jet floated up for a short time. The enlarged fragments of Fig. 11,b are shown in Figs. 11,c and 11,d. They represent the short-period jet surfacing: (c) from 15:14 on Aug. 15 to 13:50 on Aug. 16; (d) from 23:50 on Aug. 20 to 21:02 on Aug. 21.



a) b)

Fig. 9. Vertical profiles of the seawater density in Mamala Bay during the period from 13:00 on September 1 to 13:00 on September 2, 2002 (a); and trajectories of propagation of turbulent floating-up jets of deep outfalls calculated from the data of the density profiles (b).



a) b)

Fig. 10. Comparison of the parameters of jet propagation with the characteristics of the medium stratification (September 1 – 2, 2002): (a) time evolution of the level of float up of the jet *Hm* and the density gradient *d*ρ/*dz*; (b) time evolution of the initial dilution of the sewage waters and the density gradient *d*ρ/*dz*

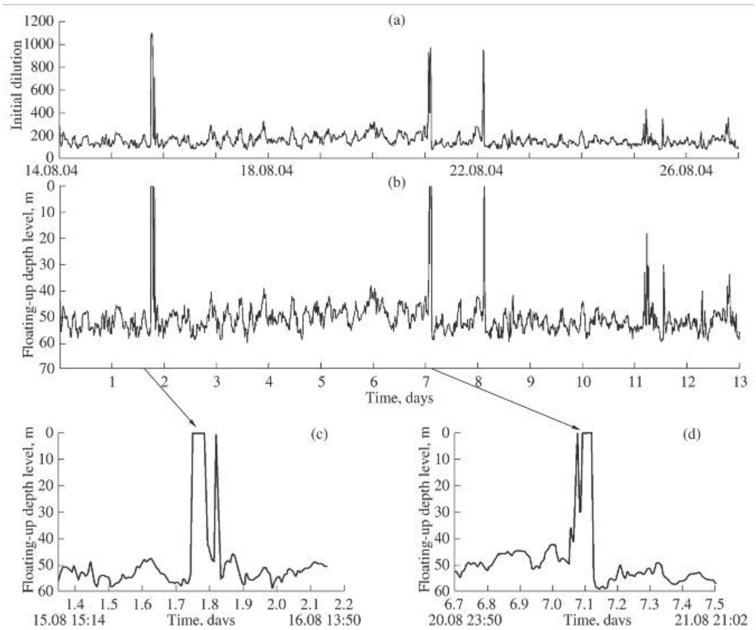


Fig. 11. Model calculations of the initial dilution (a) and the floating-up depth of the jet (b) from Aug. 14 to 26, 2004; enlarged fragments of Fig. 10,b for two short jet surfacing events from Aug. 15 (15:14) to 16 (13:50) (c) and from Aug. 20 (23:50) to 21 (21:02) (d)

6.3 Comparison of modelling and experimental data

A comparison of the parameters of the deep-water outfall discharges obtained on the basis of the experimental measurements with the results of the model calculations allows us to test whether the mathematical model applied is adequate and check the accuracy and reliability of the model estimates obtained.

Profiles of the spatiotemporal distributions of the (a) turbidity, (b) salinity, and (c), temperature of the seawater plotted on the basis of the microstructure measurements near the diffuser on September 2, 2002, from 12:15 to 15:20 are shown in this Fig. 12.

It is clearly seen from these profiles that, during the period analyzed, the discharge waters ascended to a depth of 45 m.

The levels to which the jet of sewage waters floated up calculated using the model in the period from 9:00 to 18:00 on September 2, 2002, are shown in Fig. 13,a. It is seen from the figure that, during the period from 12:00 to 16:00, the model estimate of the mean level of the floating up is equal to ~44 m, which is in good agreement with the data of the experimental measurements (~45 m). During the experiments from a research vessel on September 6, 2002 at 14:48, an anomalous spot at the sea surface was found near the diffuser. A photo of this surface anomaly taken by Professor C. Gibson is shown in Fig. 13,b.

Figure 13,c shows the outcomes of the model calculations for the same day and time period from 07:30 to 11:45. The model indicated the surfacing of the jet from 07:50 to 08:15, which is in perfect agreement with the occurrence time of the anomaly. A surface anomaly related to the floating up of the discharged waters was observed near the diffuser in 2004. A still picture of the anomaly taken on August 12, 2004 at 08:00 is given in Fig. 13,d. Similar events took place during the experiments of 2002 (Bondur et al., 2006b).

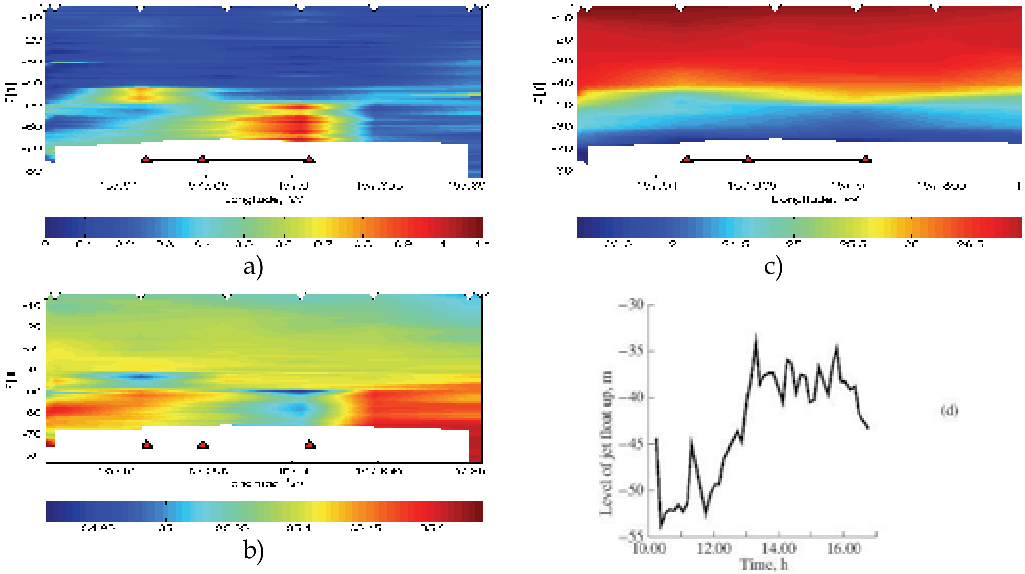

a)
c)
b)

Fig. 12. Comparison of the model estimates of the parameters of the jet with the data of experimental measurements: vertical profiles of the (a) turbidity, (b) salinity, and (c) temperature on the basis of the measurements with an MSS profiler on September 2, 2002 during the period from 14:15 to 15:20; and (d) model estimates of the depth of the sewage water jet float up in the period from 9:00 to 17:00 on September 2, 2002.

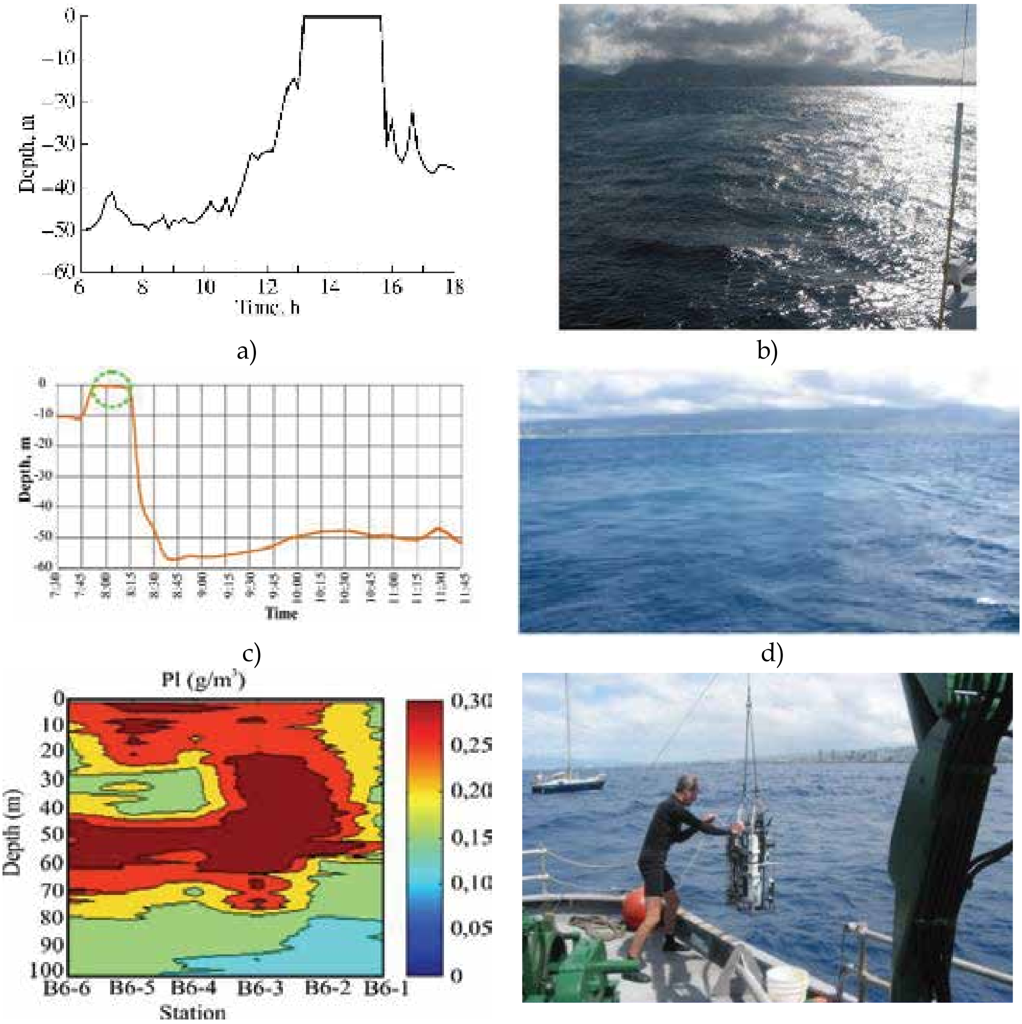
Jet floating-up was also registered by AC-9 hydrooptical sensor (see Fig. 13,f). Fig. 13,e shows an example of 2D distribution of large particle concentration obtained by AC-9 (see subsection 4.4). The analysis of Fig. 13,e have shown that the increased concentration of large particles related with the deep outfall for B6-1 – B6-7 measuring track (see Fig. 8,a) was detected at 40-70 m depths, and the jet appeared on the surface at B6-2 and B6-6 points, and max concentration near the surface in the diffuser area (B6-4 and B6-5 points).

The good correspondence of the model's estimates of the propagation characteristics of the discharged water jets with the spatial patterns of the results of the hydrophysical and hydrooptical measurements corroborates the idea of the adequacy of the description of the turbulent jet propagation mechanism in the coastal aquatic areas based on our mathematical model.

7. Conclusion

The analysis of physical features of deep plume propagation in coastal water areas has been carried out, as well as capabilities to detect the impact of these plumes on marine environment have been grounded.

Based on high resolution (0.6 – 1.0 m) satellite image processing results, it has been established that in 2D spectra of their fragments "quasi-coherent" spectral harmonics are observed. These harmonics correspond to "quasi-monochromatic" (multimode sometimes) wave systems on the sea surface, having Λ = 30-200 lengths, and ΔΛ ~ 3-5 m widening, which also can be registered by wave buoys. The analysis of physical mechanisms causing these harmonics, performed by spectra of isotherm depths, have shown that these effects are



a) b)

c) d)

e) f)

Fig. 13. Comparison of the model estimates of the parameters of the jet with the data of experimental measurements: (a) and (c) Model estimates of the float-up depth of the sewage jet in the period from 6:00 to 18:00 on September 6, 2002 (a) and from 07:30 to 11:45 on August 12, 2004 (c); (b) and (d) Photos of the surface anomaly caused by the deep-water discharge measured from a ship near the diffuser on September 6, 2002, at 14:48 by K.Gibson (b) and at 08:00 on August 12, 2004 (d); 2D profile of large particle concentration obtained by AC-9 (e); AC-9 deployment (f)

due to ultrashort internal waves generated by turbulent deep plumes in the stratified medium.

It has been established that surface anomalies which are characterized by the presence of "quasi-monochromatic" surface wave systems detected in the areas of deep outfall usually have two-lobe mitten-like shape. Its shape is quite stable, and dimensions varied between 11-23 km. Their intensity depends on outfall device operation mode, as well as by instability of hydrodynamical and meteorological modes of the studied water areas and tide influence.

As a result of high resolution (1-4 m) multispectral satellite image processing, there have been detected small-scale hydrooptical anomalies caused by intensive deep outfalls, and theirs geometry has been determined (5-20 km max). The comprehensive analysis of satellite image processing results and sea truth data has shown that the dimensions and propagation directions of these anomalies almost coincide with spatial distributions of hydrooptical parameter fields. This indicates the adequacy and efficiency of this method to study deep wastewater outfall impact on coastal water areas.

The processing of radar satellite imagery was carried out using specially developed methods providing online computer-aided detection and classification of surface anomalies. The comprehensive analysis of this processing results together with sea truth data have allowed us to detect the anomalies of high frequency surface waves (comparable with radar wavelength) in the areas of deep outfalls, to determine their variability depending on meteorological and hydrodynamical modes in the water area.

The model developed was used to estimate the parameters of a floating-up jet of deep wastewater discharge from Sand Island into the basin of Mamala Bay (Hawaii) depending on the season and discharge operation mode. The estimates of the float-up depths of the jet and the initial dilution of the jet were estimated on the basis of model calculations using experimental data on the vertical profiles of the water temperature and salinity under the actual conditions of stratification in the study region at various times. It is shown that the further propagation of the wastewater jet (first of all, at the depth of floating-up) depends on tidal events and internal waves generated by tides. The model estimates of the parameters of the wastewater discharge were compared with the results of experimental measurements. Good agreement was found, which indicates that the physical mechanisms of the propagation of turbulent jets in a stratified medium are adequately described by the model.

The results from the Mamala Bay monitoring (Hawaii, USA) are also confirmed by the data obtained in the Black Sea water areas near Gelenjik city (Russia).

Taking into account the big volumes of wastewater discharged into the water area of Mamala Bay (~ 70 mln. gallons/day), the presence of significant quantity of polluting substances (despite of good treatment system) and high requirements to seawater conditions in recreational zone of Honolulu city, some measures aimed to decrease anthropogenic load on the ecosystem of Mamala Bay are proposed based on the results of satellite monitoring.

1. In case of unfavorable conditions (tides, onshore current and wind directions (to Waikiki Beach), absence of thermocline), it is expedient to reduce the discharge rate as much as possible by accumulating wastewater in special WWTP reservoirs.

Under favorable conditions (ebbs, southern and southwestern directions of currents, south and southwest winds, expressed thermocline) it could be advised to increase the discharge rates since this is the best circumstances for their disposal.

2. To provide reliable information on favorable and unfavorable conditions and on water area environmental situation, it is necessary to maintain permanent monitoring of major parameters in Mamala Bay water area (current fields, CTD-measurements, wind speed and direction, air temperature, etc.), as well as to perform permanent aerospace monitoring by means of processing and analysis of remotely sensed data comparing it with the results of in-situ measurements.
3. Increase the density of wastewaters for their better disposal, e.g. by adding salt or diluting with seawater. Decrease volume of discharged waters in the coast part by

closing a part of diffuser ports at its north side. Increase the level of wastewater treatment by applying new technologies.

Such nature-preserving measures can be undertaken also for other water areas under intensive anthropogenic influence.

The presented results confirm the efficiency of aerospace methods and technologies, as well as methods of mathematical modeling deep turbulent plume propagation to monitor anthropogenic impacts on coastal water areas.

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8

Intelligent Photonic Sensors for Application in Decentralized Wastewater Systems

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1. Introduction

The generation and treatment of wastewater is considered a serious ecological, economical and technical problem (Bourgois et al., 2001); (Richardson, 2003); (Richardson, 2004); (Savage & Diallo, 2005); (Bartrand et al., 2007). There have been several reviews published concerning the instruments and methods of monitoring the contamination of water and detection of contaminants in water samples (Moorcroft et al., 2001); (Nakamura & Karube, 2003); (Dabek-Zlotorzynska & Cello, 2006); (Dabek-Zlotorzynska et al. 2008).

Recent publications on detection of nitrate and nitric oxides in water include (Cho et al., 2001); (Ensafi & Kazemzadeh, 2002); (Sun et al., 2003); (Wen & Kang, 2004); (Bates & Hansell, 2004); (Biswas et al., 2004); (Palaniappan et al., 2008); (Sivret et al., 2008). A method of detecting sulphide in water was presented (Ferrer et al., 2004), as well as one for chlorite (Praus, 2004), other inorganics (Hua & Reckhow, 2006); (Masar et al., 2009) and acidic drugs (Basheer et al., 2007). The sensors of metallic contaminants in water and their performance have been reported for the case of iron (Pons et al., 2005), arsenic (Toda & Ohba, 2005), chromium (Tao & Sarma, 2006) and other metals (Masàr et al. 2009).

New organic contamination detection methods and instruments have been widely reported in recent literature (Lucklum et al., 1996); (Bürck et al., 1998); (Rössler et al., 1998); (Yang et al., 1999); (Scharring, 2002); (Yang & Chen, 2002); (Yang & Lee, 2002); (De Melas et al.,2003); (Fernàndez-Sànchez et al., 2004); (Kamikawachi et al., 2004); (Sluszny et al., 2004); (Falate et al., 2005); (Pons et al., 2005); (Mauriz et al., 2006); (Rodriguez et al., 2006); (Tao & Sarma, 2006); (Jeon et al., 2009). Optical sensors for bacteria detection and quantification in water have been reported (Ji et al., 2004); (Zourob et al., 2005); (Nakamura et al. 2008).

1.1 The configuration of wastewater treatment systems

The major sources of wastewater can be classified as municipal, industrial and agricultural. Wastewater can be treated in wastewater treatment plants (WATP) or in decentralized wastewater treatment systems (DEWATS) (Jo & Mok, 2009). Wastewater can be described using physical properties and by a list of chemical and biological constituents which should be precisely specified (Muttamara, 1996). The physical properties of wastewater are commonly listed as color, odor, turbidity, solids content and temperature. The wastewater treatment and disposal commonly depends on water contamination with suspended solids, biodegradable organics, pathogens, nutrients, refractory organics, dissolved inorganic solids and heavy metals. The heavy metals are particularly present in industrial wastes. The typical examples of refractory organics are surfactants, phenols and pesticides. While phenols are present in industrial wastes, pesticides in agricultural wastes, surfactants are common in households' wastes. The surfactants (Abdel-Shafy et al., 1988) and oils tend to resist conventional methods of wastewater treatment.

The properties of wastewater in the treatment process have to be monitored, particularly before the effluent water is discharged to the environment. The commonly examined parameters of wastewater before, during and after treatment in WATP are: pH, electric conductivity (EC in μS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total kjeldahl nitrogen (TKN mg/l), total organic carbon (TOC), total suspended solids (TSS), and also bacteria presence (E. Coli- number/100ml) (Thomas et al., 1997). Users of WATP run regular tests for those parameters.

DEWATS are intended for recycling domestic wastewater from individual households, community plants and small industrial type systems producing effluent with similar characteristics to domestic wastewater (Qadir et al., 2010). The objective of their operation is efficient removal or conversion of the various types of pollutants that are present in wastewater (Shirish et al., 2009). A typical DEWATS configuration is presented in Table 1.

Treatment	Device	Function
Primary	Settling tank	Initial separation solids and liquid. Solid matter or sewage disintegration by bacteria.
	Septic tank	
	Anaerobic baffled reactor	
Secondary	Mechanical filter for example: sand or membrane.	Filtration of wastewater to the acceptable discharge standard.
	Horizontal planted filter: • filter media: pebbles with top layer of sand, • plant cover: Canna Indica and Arundo Donax.	
Finish	UV electrically powered filter	Reduction of bacteria and virus count.
	Open collection tank	In the regions with high solarization the collected water is naturally UV-filtered.
	Open polishing tank	

Table 1. Example of typical configuration of DEWATS

Domestic wastewater can be divided into grey and black wastewater. The grey wastewater may be used directly for undersurface irrigation, when the irrigation does not cause formation of ponds. It is recommended however that grey water should be treated before use and that its contamination by surfactants should be tested. When the level of surfactants in grey wastewater is high the discharge should be directed to sewage. The oil presented in grey wastewater can block up the filters, so their condition also should be tested. The common way of treatment of grey and in some cases black wastewater is sedimentation with microbiological disintegration in compact devices and mechanical filtration. Planted vegetation is used sometimes for additional filtration. The UV light disintegration of pathogens is also recommended as finishing treatment.

1.2 Sensors of parameters of liquids

There are many types of sensors that can be used for water and liquid monitoring, including a wide range of fiber optic sensors with chemical or biological sensitive layers, and electrochemical sensors that use fuel cells (Cusano et al., 2008). Under development are sensor devices that could be used for wastewater monitoring: pH meters, conductivity meters (EC), sensors for selected metal ion concentration, turbidity, liquid and sludge level meters, flow meters, sensors of particle presence in flowing liquid and biosensors of aerobic activated sludge organisms (Fazalul Rahiman & Abdul Rahim 2010) (Holtmann & Sell, 2002). The suspended solids concentrations and size distribution and particle weight can be determined from turbidity measurements. The metal ion concentrations of dissolved oxygen and carbon dioxide can be measured by using sensitive layers deposited on fiber tips or inside of capillaries where they are optically monitored. The wastewater contamination with toxic colony of micro organisms and BOD can be detected using fluorescence methods that include adding a sensitive fluorescent liquid to the examined sample or by the immobilization of a microbial layer on an amperometric oxygen electrode. The composition of wastewater can be also monitored using near-infra-red (NIR) spectroscopy, but this technique requires a laboratory setup and the set of reagents. Water contamination can be also analyzed indirectly in the form of gas with the use of a chemical nose which is a matrix sensor with integrated signal processing. There are sensors array systems intended for monitoring volatile components of wastewater. In more advanced chemical noses the wastewater sample is turned into vapor phase before the measurement is performed (Bourgeois et al., 2003). In such systems the detector of the principal contaminating component is used as the classifier of wastewater pollutants. The problem of implementation of sensors in wastewater monitoring is mainly the cost of keeping the sensor running or the time needed for examination and calibration.

1.3 The design objectives of DEWATS

Apart from technical aspects, the efficiency and the costs of the purification of wastewater, which include the cost of wastewater examination, require serious consideration (Rulkens, 2008). The simple DEWATS configuration does not include sensors for discharge monitoring, but as mentioned, the surfactants contamination and oil disintegration should be tested. The operation of DEWATS should not require constant samples examination in a laboratory. Therefore, DEWATS users need simple in use, low cost and fast sensing methods for in-situ initial qualification of water treatment and discharge (Vanrolleghem & Lee, 2003). Such methods would use sensors operating in a continuous mode without use of reagents, and would feature simple or automatic head cleaning and regeneration. The sensors for DEWATS have to be low cost in construction and operation and they have to enable monitoring of surfactants presence and give a clear answer if the discharged water is acceptable from environmental control point of view. Such requirements can be met by physical methods of measurement using light or the electric current.

2. Intelligent photonic sensors for wastewater treatment monitoring

In this work we present intelligent photonic sensors that can be used for monitoring of wastewater treatment. These sensors work on the principle of optical intensity changes that take place in dynamically forced measurement cycles. The sensors examine simultaneously many liquid parameters which are processed in artificial neural networks (Borecki et al., 2008a). The first type of sensors monitors signals from a drop forming during emerging and after emergence of an optical fiber from the examined medium (Borecki, 2007). The second type of sensors uses a fiber optical capillary in which the phase change from liquid to gas and again to liquid is forced by local heating while the propagation of light across the capillary where the liquid changes phase is monitored (Borecki et al., 2008b).

2.1 The examined liquids

To evaluate the proposed systems we used several liquids: still water, sparkling water, fresh edible oil, spoiled edible oil and grey wastewater including in its composition commonly present domestic discharge contaminants. We examined the still and sparkling waters coming from this same source and producers. The sparkling water was saturated with carbon dioxide. The detection of dissolved CO_2 is based on the measurements of differences of the solubility of gases in water. Values of gas solubility in water are presented in Table 2.

Gas	Solubility (ml/L)
Nitrogen	16.9
Oxygen	34.1
Methane CH_4	35
Carbon Dioxide CO_2	1019

Table 2. Examples of gas solubility in the water at 20°C

To simulate domestic grey wastewater with controllable composition we used water with suspended solids (carbon powder), biodegradable organics (rapeseed oil, milk, fats), nutrients (sugar, starch), and refractory organics (surfactants) and also dissolved inorganic solids (some components of powder milk). We did not include in the composition heavy metals and pathogens, but the pathogens can arise in the presence of milk, yogurt and sugar, Table 3.

Type of contaminants	Concentration of contaminant
Carbon powder	75 mg/l
Biodegradable surfactant	5ml/l
Rapeseed oil	10ml/l
Proteins with milk acid bacteria (Actimel)	1.25 ml/l
Proteins with fat (Powder milk 3.2% of fat)	1g/l
Starch (Flour)	1g/l
Sugar	1g/l

Table 3. Composition of the examined grey wastewater

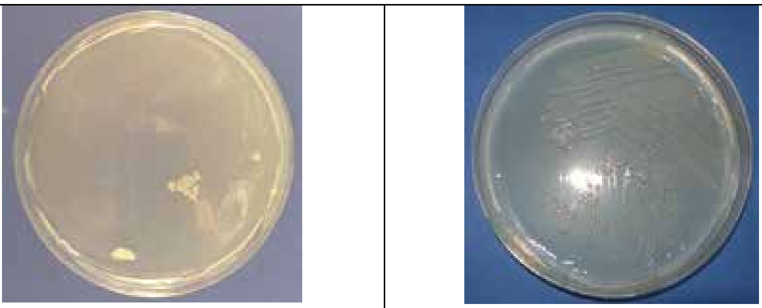
The composition was treated for a few days in a still tank with a biological activator. We used as activator a 1ml/l solution of 0.5 tablet which includes 4*108cfu of nonpathogenic bacteria and enzymes that can disintegrate proteins, starch, oils, fats, papers and surfactants.

After dissolving, the tablet works like a mixture of soda and vinegar. Our still tank was kept at 26°C and had a volume of 5 liters and a height of 30cm. The sample for examination was probed from the middle part of tank using a pipette. The changes in the liquid in 4 days of probing in terms of pH and capillary action, which was measured in a glass capillary with a diameter of 536μm, are presented in Table 4. The visualization of bacteria growth during the treatment is presented in Fig. 1.

Treatment [day]	pH	Capillary action in [mm]
0	6.908	25.6
1	7.925	25.3
2	7.912	27.0
3	8.168	26.6

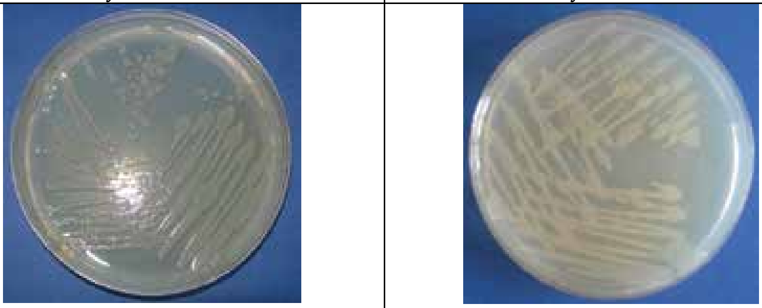
Table 4. pH and capillary action of grey wastewater in the function of treatment holding time

The measured pH of the sample 1 day after preparation was about 8 and remained stable during the following days, while initially the pH of the water was 7.0. The capillary action remained stable at the average level of 26mm, while the capillary action of clear water was about 39mm



0 days treatment

1 day treatment



2 days treatment

3 days treatment

Fig. 1. Visualization of bacteria growth as function of treatment holding time

The microscopic examination in the following days showed that our gray wastewater has slightly increased number of bacteria. Therefore, we see that after 3 days of treatment our gray wastewater which sediments in tank quite effectively, was not fresh drinkable water.

2.2 Experimental setup

2.2.1 General description

We used two experimental setups, both with intelligent optoelectronic multi-parametric signal detection (Borecki et al., 2008a). Both examined sensors used light intensity measurements in forced measuring cycles and they used electrically controlled actuation to generate time-dependant information (Borecki et al., 2010). In their construction we used to the extent possible commercially available components.

The light source, and detection hardware were the same in both constructions. The heads were optically connected using large core SMA optical connectors. As light source we used a fiber coupled laser source S1FC635 from THORLABS that was coupled to the sensing head with a multi-mode optical path-cord finished with FC connectors and FC to SMA mating sleeves. The S1FC635 enabled light power stabilization and adjustments of power in the range from 0.01mW to 2mW. We eliminated the effect of the ambient light by modulating the probing light with 1kHz by connecting electrically a DG2021A function generator to the modulation input of S1FC635. The scheme of the light source is presented in Fig. 2.

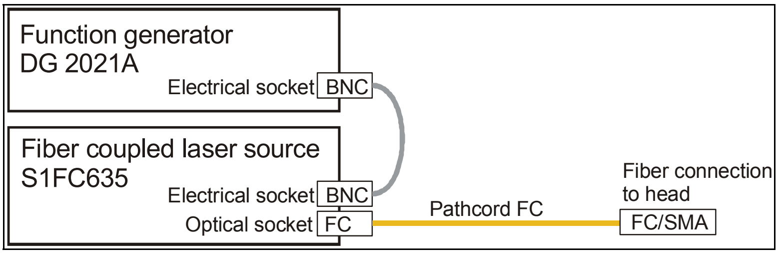


Fig. 2. The light source used in experiments

In our experiments we used the optical signal from a S1FC635 LD at a level from 0.01 to 0.2mW. The signal was transmitted almost without losses to the head by a SMA socket. The presented light power coupled into large core fibers could be also realized using properly selected LED diodes powered from an electric driver which consisted of a laboratory power supply that had precise output current settings and a transistor switch connected to the generator.

The detection hardware consisted of an optoelectronic interface, a data acquisition system, an electric actuation system and a PC with software, as shown on Fig. 3.

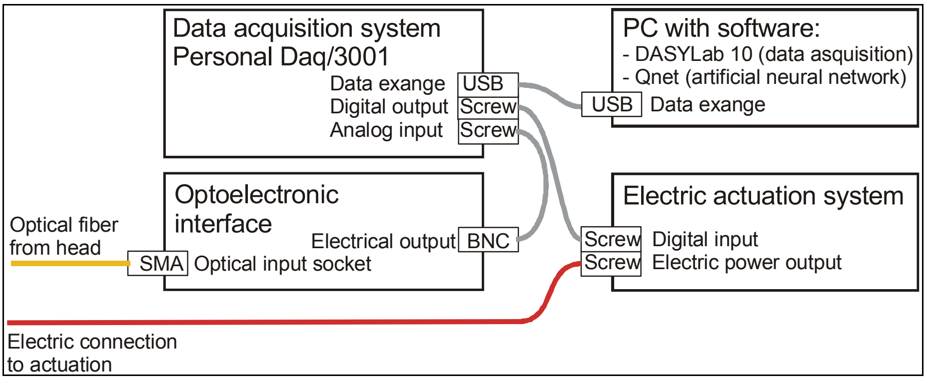


Fig. 3. Scheme of the detection hardware

The optoelectronic interface converted the intensity of amplitude modulated light into an electric signal. First the light was converted to the electric signal by a photodiode that was integrated in a trans-impedance circuit OP301. Then, all the components of the electric spectrum that were not in the modulated band were filtered with the UAF42 circuit. Next, the sensed changes of the modulated light intensity were demodulated with an AD536 true RMS detector. The interface was sensitive for the changes of the modulated signal slower that 5V/0.01s. The most expensive elements of the optoelectronic interface were the SMA socket (about 16EUR distributor's price) that was positioned mechanically directly above the OP301 (50EUR).

The signal from the optoelectronic interface was fed to the data acquisition system that read analog signals and converted them to the digital form proper for processing in the data acquisition software. We used DASYLab software with two scripts. The first DASYLab script was developed for data acquisition and the second was aimed for data classification. The data were analyzed with 0.1second time base and were observed and converted to the form required in the artificial neural network (ANN) Qnet microcontroller with embedded software. We used ANN that was in the form of multilayer perceptron, because this configuration showed its high usability in signal classification in sensors technique, (Borecki & Korwin-Pawlowski, 2010).

2.2.2 Fiber optic setup for fiber drop analysis (FDA)

The first sensing setup consisted of a mini-lift holding an optical fiber optic with a bare tip as a measuring head. This setup is presented in Fig. 4 and we used it for intelligent fiber drop analysis.

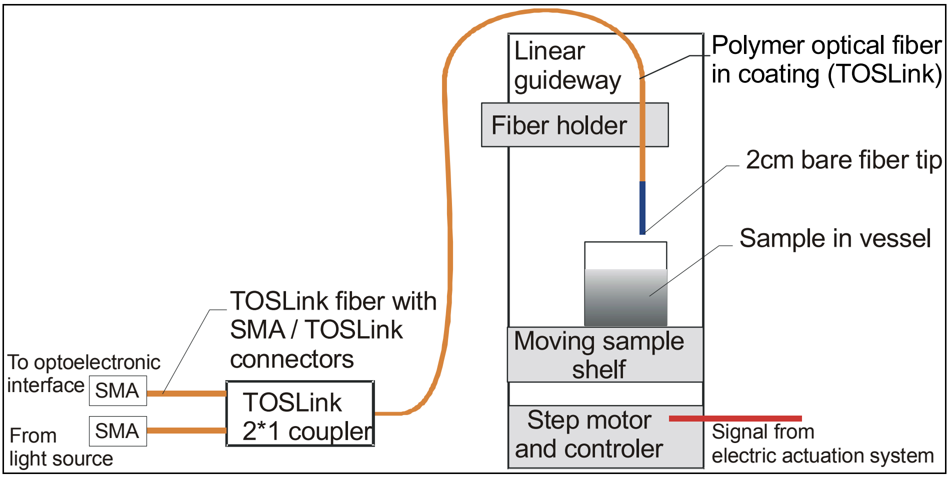


Fig. 4. Scheme of mini-lift sensing setup used for fiber drop analysis

In this setup we used a linear guideway type MLA0373-5HK1SKK from Wobit with a length of 50cm powered by a 10W step motor 57BYGH804. The guideway and optical fiber of the head were mounted on the wall for stability. On the guideway we fixed a vessel with a sample of a volume of 100ml. We controlled the movement of the liquid sample in the directions up and down with a tolerance of 0.1mm by using a data acquisition system and software. This construction provided a stable optical path that resulted in a more repeatable signal than the configuration with a moving fiber and a fixed vessel. We configured the optical path using slightly modified TOSLink standard elements. We found that present polymer optical fibers can have their coating stripped easily from the fiber without damage being inflicted to the cladding or to the core. The sensing arm was one half of TOSLink pathcord type T-T from Vitalco PRC cut in half with stripped coating tip on 2cm length. The connections from light source and to the optoelectronic interface were made from HC302-200 Clicktronic pathcord cut in a half with mounted SMA connectors on the cut tips.

We have also considered using pathcords from different producers and found them working not as well with TOSLink coupler, but we found only one type of TOSLink coupler available on the market. Inside the coupler there were four fibers with slightly smaller diameter than ½ of the TOSLink fiber which we put together on our head arm and each two fibers were connected with the input and output arms as is presented in Fig. 5. Therefore, the coupler was in fact a divider and gave us the coefficient of light coupling from the source to the detection lower than 25%. We also evaluated the SMA BFL48-600 pathcord from Thorlab which had a core diameter 600μm, cladding diameter 630μm, coating diameter 1040μm and numerical aperture (NA): 0.48 ± 0.02 and a multimode FC pathcord with core 62.5mm for making the asymmetrical coupler presented in Fig. 5.

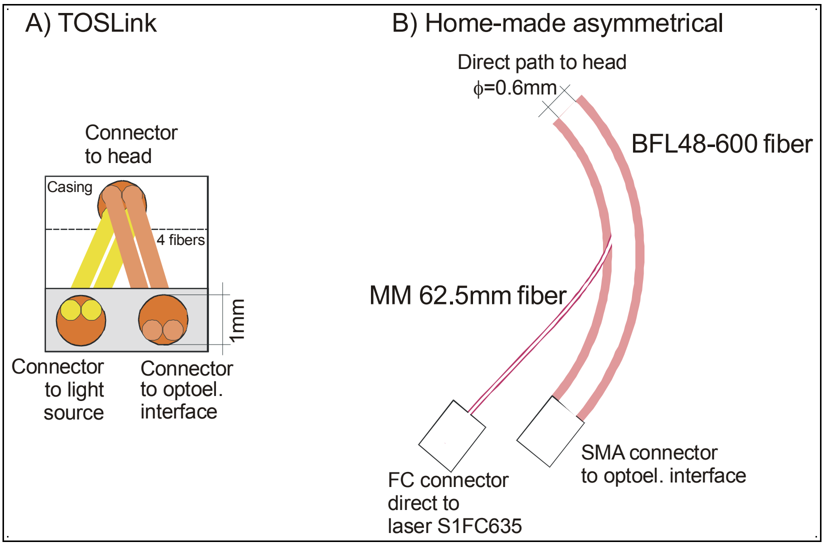


Fig. 5. The two variants of couplers: A) – TOSLink, B) – Asymmetrical

The asymmetrical coupler had a coefficient of light coupling from the source to the detection equal 43%, which was much higher than in the TOSLink construction, but the construction with only TOSLink elements had still sufficient light power output and, moreover, the light power balance in TOSLink did not decrease unacceptably when the LED light source was tested. With the LED light source the asymmetrical coupler made from polymer fiber presented in (Borecki, 2007) is recommended.

2.2.3 The setup for liquid-gas phase change measurements

The second sensing setup consisted of a head base, optical fibers, a miniature heater and a disposable capillary. This setup is presented in Fig. 6 and we used it for intelligent liquid-gas phase change capillary measurements.

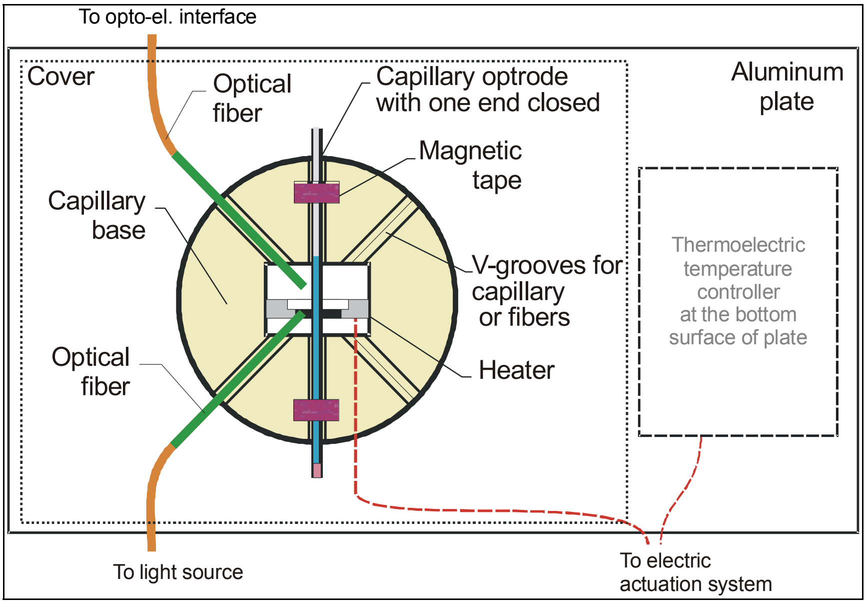


Fig. 6. Scheme of capillary liquid-gas phase change sensing setup

In this setup we used capillaries TSP700850 from Polymicro Inc. and BFL48-600 optical fibers with outer diameters of cladding similar. An important feature of the capillary probe was that the top end of the capillary was blocked with the operator's finger after the sample was drawn and the bottom end contacted with liquid was blocked with modeling clay. This prevented any sample spilling and ensured a safe transfer from the place of sample drawing to the point of examination. The capillary had the length of 6cm and after introducing the sampled liquid by capillary force to the length of about 20mm, modeling clay was inserted to a length of a few millimeters to act as a stopper.

We used a SMA BFL48-600 fiber-tipped pathcord cut in a half. The stripped ends of the fibers were mounted with mechanical clamps on the capillary base that was made from steel with the tolerance of 2μm. The base was mounted on top of an aluminum plate. A replaceable cover was put over the plate to prevent changes of heat transfer due to uncontrolled air movement. On the bottom of the plate a thermoelectric temperature controller was mounted to stabilize the temperature of the plate with an accuracy of 0.5°C.

The heater was made in thick film technology. The heating area was 1mm×3mm and the heater could dissipate 10W in 60 second without degradation, with 6 minutes of stabilization time required between temperature steps. The heater could generate a bubble in the liquid filling the capillary above the middle or the edges of the heating area with the bubble always moving towards the open end of capillary. Therefore, to avoid false measurement results the observations were done above the edge of the heater closer to the open tip of capillary.

2.3 Experimental results of fiber drop analysis (FDA)

The scheme with a mini-lift and a head with a bare POF fiber generated repeatable time-domain signal waveforms. For example, during the examination of still water repeated 10 times it gave signals presented in Fig. 7. The signal can be analyzed considering two dynamic phases of the sensing head moving down (submerging) and up (emerging). When the head during submerging crosses the liquid level the reflected signal decreases. The signal drop depends on the indexes of refraction of the liquid and the fiber and on the turbidity of the liquid. The signal decreases during first part of head emerging cycle. When the head comes out of the liquid it takes with it a drop of the liquid. The signal behavior next depends on the liquid's parameters as: density, viscosity and surface tension related to the fiber material which in this case should be not wetting. Probing of still water results in formation of a drop that increases in volume and lasts for about 3 minutes when it comes off. After that the signal returns to its initial level.

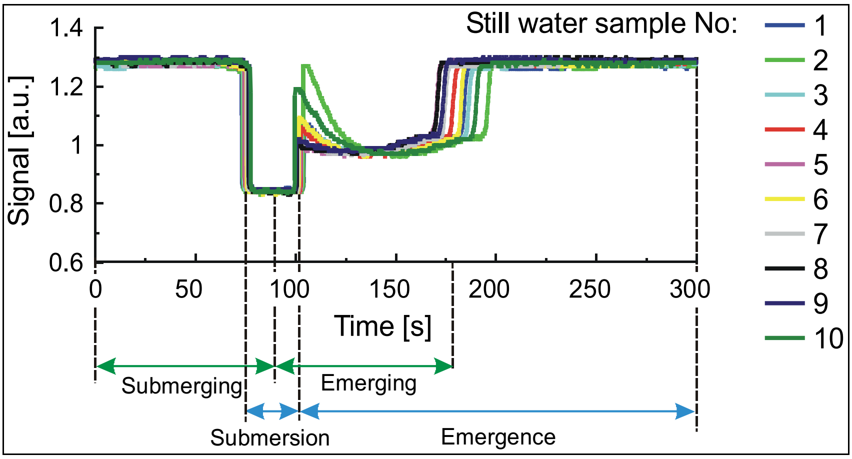


Fig. 7. Signal collected in FDA for still water samples

In Fig. 8 is presented the signal collected from the solution of milk power in water at the concentration of 500mg/l.

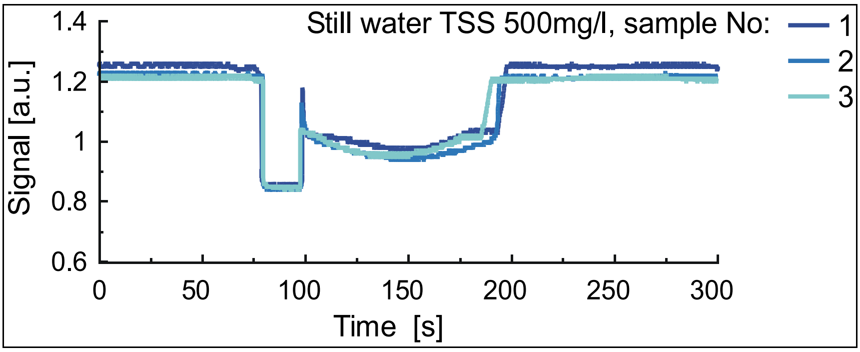


Fig. 8. Signals collected in FDA for samples of milk powder in still water at the concentration of TSS = 500mg/l

Clearly, the signals presented in Fig. 7. and in Fig. 8. do not differ significantly. To simulate closer the grey wastewater we added out-of-date edible refined rapeseed oil (without chemical modifications) to the examined solution. We observed a 1mm thick coat of oil forming on the water surface. The collected signals are presented in Fig. 9.

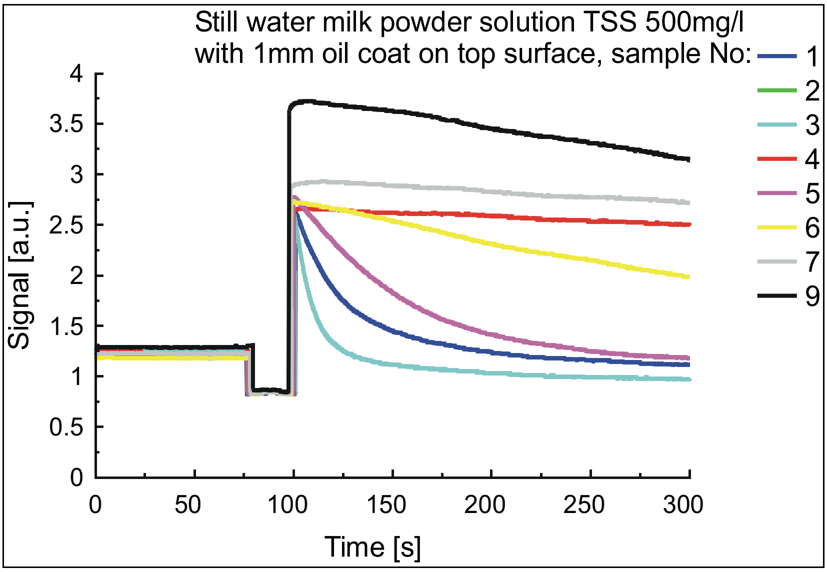


Fig. 9. Signal collected in FDA for samples prepared of still water with milk powder in concentration of TSS = 500mg/l and covered with 1mm out of date oil coat

The modification of the liquid sample with out of date oil introduces big differences between the collected signals. The signals collected for liquid covered with 1mm thick coat of fresh refined rapeseed oil are presented in Fig. 10.

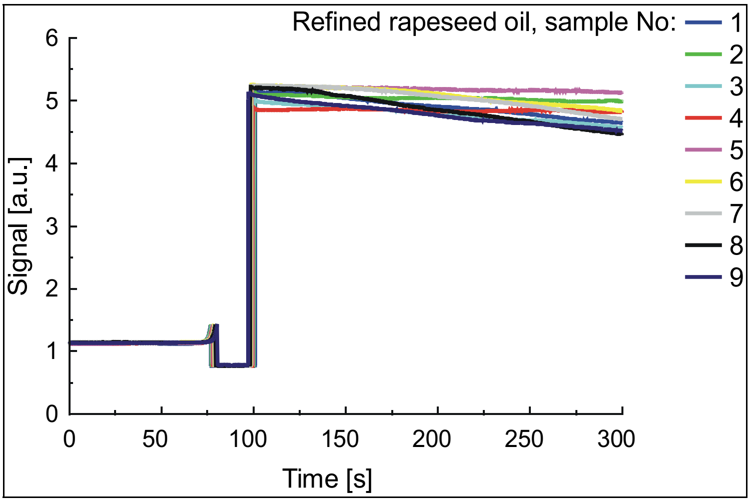


Fig. 10. Signal collected in FDA for samples prepared of fresh refined rapeseed oil

The comparison of the characteristics from Fig. 9 and Fig. 10 leads us to the conclusion that the signals from FDA for the samples of liquid with layer of fresh refined rapeseed oil are repeatable, contrary to the signals collected from the layer of out-of-date oil on the surface of water. We evaluated also the influence of a surfactant as water pollution agent. The characteristics collected for a 5ml/l solution of biodegradable kitchen surfactant are presented in Fig. 11.

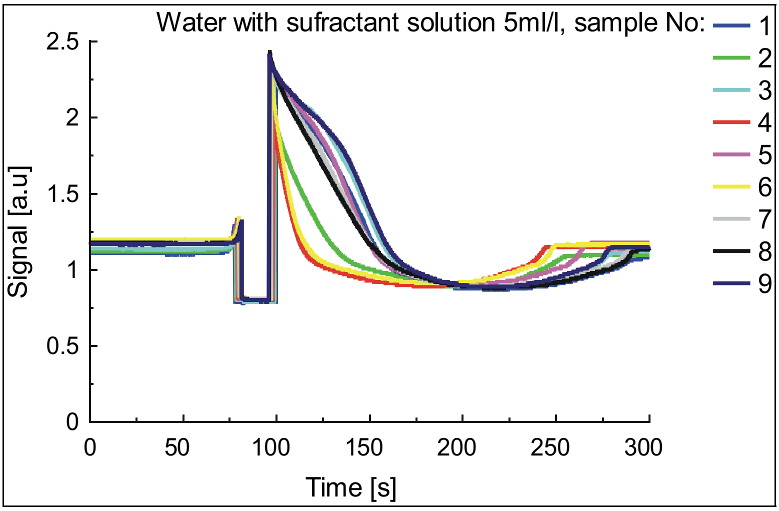


Fig. 11. Signal collected in FDA for water with biodegradable kitchen surfactant 5ml/l solution

The last individual agent we examined that could be normally present in the wastewater was carbon dioxide in the form of gas saturating bottled sparkling water. The following samples were taken from the bottle in specified time in a period of about 6 minutes with the time of opening the bottle was labeled 0min, as shown on Fig. 12.

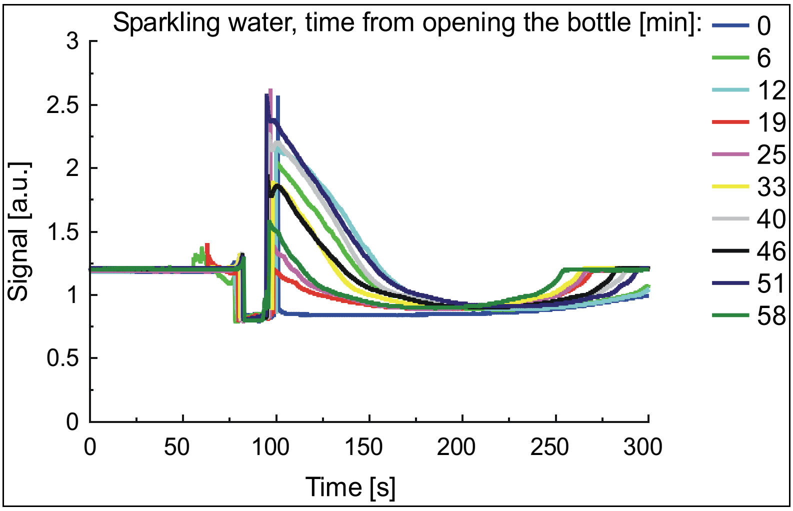


Fig. 12. Signal collected in FDA for sparkling water

An observation can be made that in the presented method the water surfactant solution with a concentration of 5ml/l and the sparkling water results in similar signals versus time dependences. Similarly, washing objects is more efficient when using water with surfactant or sparking water than still water.

Finally, we did tests with grey wastewater that was stored in a still tank for a few days. The collected data are presented in Figs. 13-15.

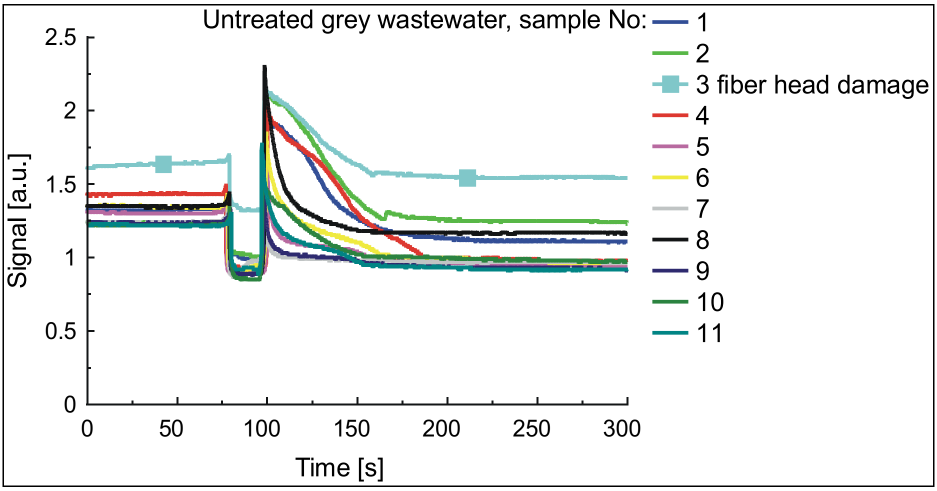


Fig. 13. Signal collected in FDA for grey wastewater just after preparation

The data collected from raw grey wastewater just after preparation is presented in Fig. 13. During that experiment we damaged the fiber head while cleaning it with a piece of tissue. The damage was visible in the fiber cladding. The signal collected for next sample has lover dynamics and increased level, which can be explained with changes in optical path parameters due to fiber damage. The way to restore the head was simply to cut off the damaged section, strip another fiber section and re-position the fiber tip. After this procedure we collected the signals from the next samples. The signals collected for grey wastewater that was treated in still tank for 1 day are presented in Fig. 14.

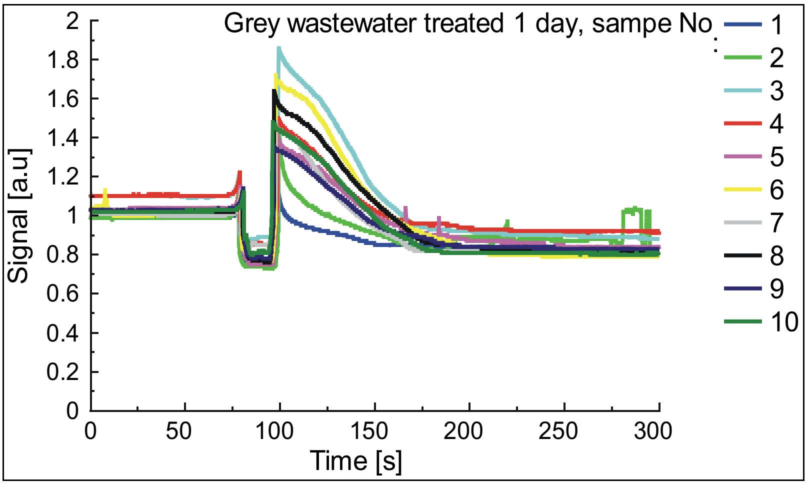


Fig. 14. Signal collected in FDA for grey wastewater that was treated in still tank for 1 day

The result of two days sample treatments is evident from comparison of data from Fig. 13 to Fig. 15. Firstly, the examined wastewater just after preparation is not a homogeneous mixture. This mixture stabilizes its parameters, but comparing Fig. 15, Fig. 7 and Fig. 11 gives us information that the presented treatment does not produce clear water which is in accordance in biological examination shown on Fig. 1 and in Table 2. It is probable that the

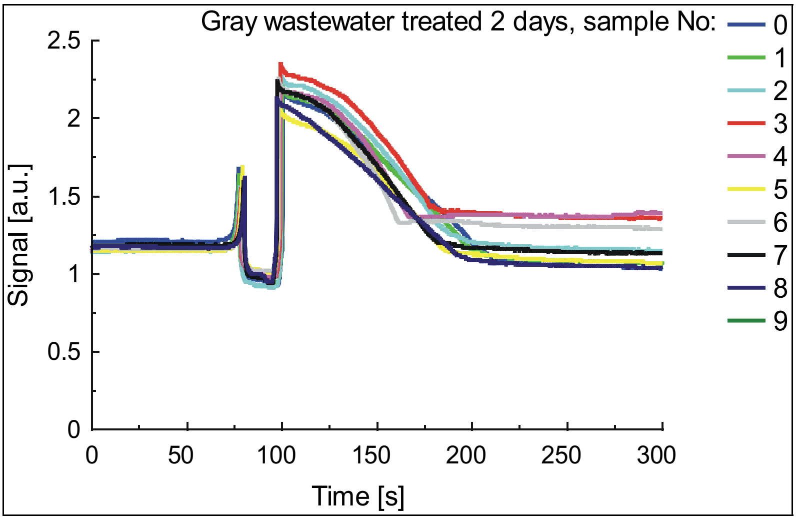


Fig. 15. Signal collected in FDA for grey wastewater that was treated in still tank for 2 days

biodegradable surfactant as suggested by its producer is not as quickly biodegradable as we may wish. After four days we observed a layer of coat on the surface of the grey water, the signals collected during this examination are presented in Fig. 16.

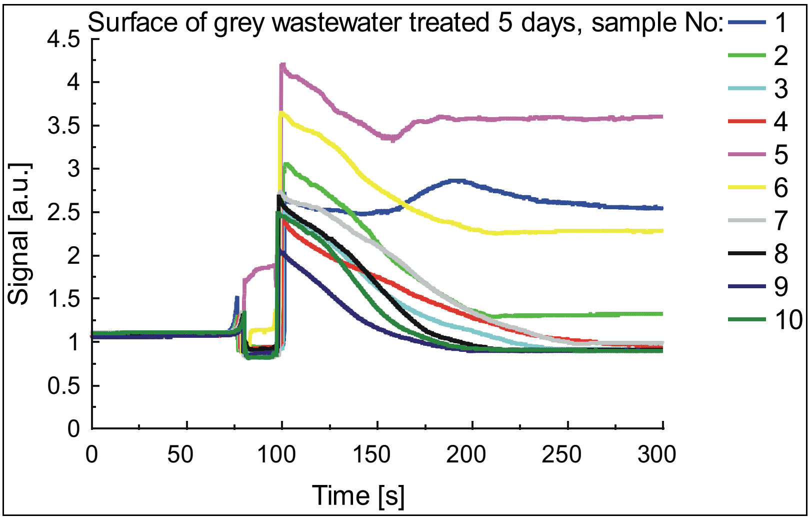


Fig. 16. Signal collected in FDA of grey wastewater surface that was treated in still tank for 4 days

The coat looked like thin ice surface with slightly yellow color. The exact observing of the coat resulted in conclusion that its structure was not uniform and it could be easily break into parts. The signals in measurement cycles were not repeatable in the presence of the coat. Sometimes the measurement cycle broke the coat and during the following measurements we effectively examined wastewater without coat (samples No. 2, 3, 4, 7, 8, 9, 10). Interestingly, the signal for sample with coat looked somewhat similar to the signal for samples with an oil layer on the water surface (Fig. 9).

2.4 Experimental results of capillary liquid-gas phase-change analysis

The sensing setup enabled the measurement of the reflected and scattered signals, but we present and use only the reflected signals. The signals were collected for 120s for liquid locally heated with 10W until the gas bubble was created. The signals collected for still water are presented in Fig. 17

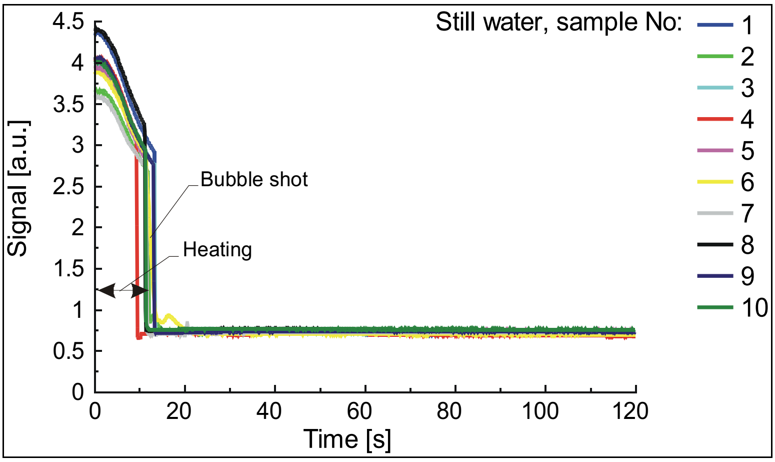


Fig. 17. Signal collected in capillary system for still fresh water sample

The signal during local heating first decreased slowly, which can be explained by the lowering of the refraction index of liquid. Next, we examined the water with the surfactant solution in volume concentration of 5ml/l, as shown on Fig. 18.

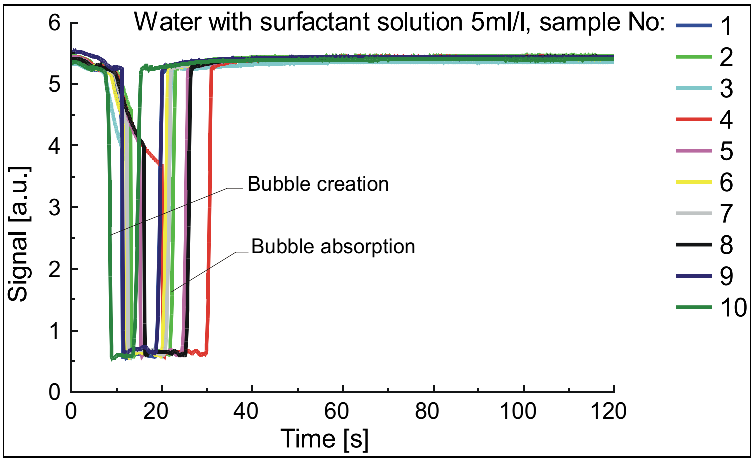


Fig. 18. Signal collected in capillary system for water with surfactant solution of 5ml/l

The created bubble in the capillary filled with water caused a shot of a drop of water out of the capillary; the added surfactant modified the situation significantly causing liquid motion slower and vapor phase absorption in the liquid. Also, the time of creation of the bubble varied when the surfactant was added to water and the initial signal level changed.

The last examination we did with the capillary system was of the grey wastewater that had been stored in a still tank. The data collected are presented in Figs. 19-21.

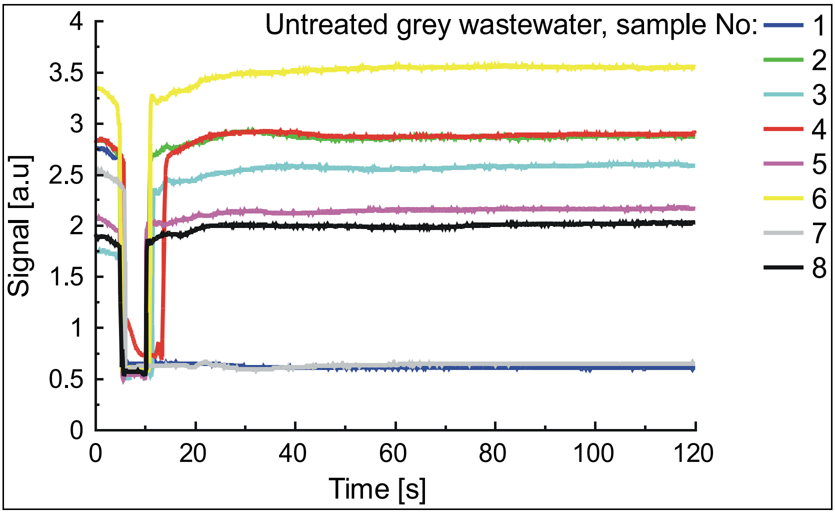


Fig. 19. Signal collected in capillary system for untreated grey wastewater

The time when the bubble formed and when a drop of water shot out of the capillary in the case of unprocessed grey wastewater was very repeatable and was one half of the time of bubble formation and drop shot-out for the clear water previously examined.

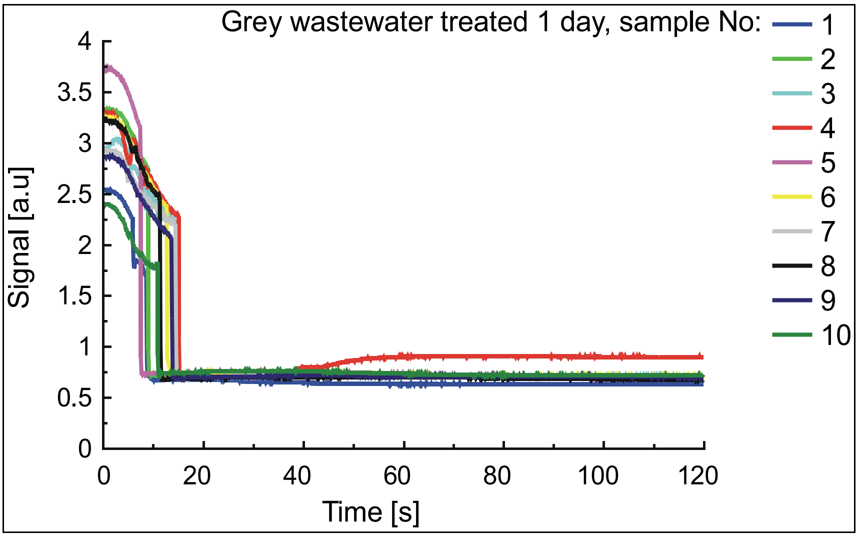


Fig. 20. Signal collected in capillary system for grey wastewater treated 1 day

The signals after one day of treatment, presented in Fig. 20, had lower initial levels than for clear water (Fig. 17), but no bubble shoot out was observed.

The signal from the sample No 10 presented in Fig. 21 has a sudden peak at 270s, the effect of local impurities of wastewater. In all other samples on Fig 21 the shape of signals versus time is similar to that measured for clear water Fig. 17.

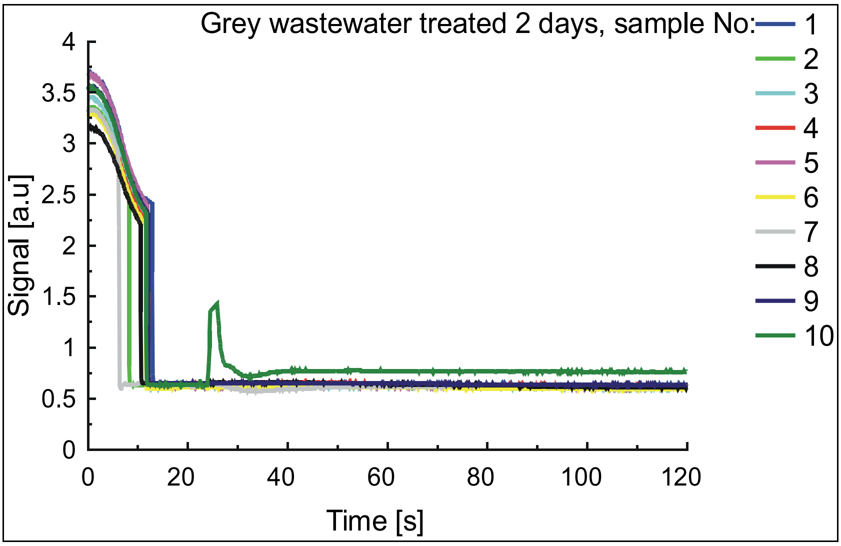


Fig. 21. Signal collected in capillary system for grey wastewater treated 2 days

2.5 Water classification with ANN in photonic systems

We examined sequentially samples of liquids of the same composition with the fiber optic FDA and the capillary system. For both types of the measurement systems our intention was to classify the water according to 5 classification states: clear water, water with surfactant solution of concentration 5ml/l, grey wastewater without treatment and after 1 and 2 days of treatment. The corresponding outputs of the ANN are given in Table 5.

Water state	No. of ANN output				
	1	2	3	4	5
Clear water	1	0	0	0	0
Water with surfactant	0	1	0	0	0
Grey wastewater – raw	0	0	1	0	0
Grey wastewater - 1 day treated	0	0	0	1	0
Grey wastewater - 2 day treated	0	0	0	0	1

Table 5. The outputs of ANN for FDA water classification

The inputs of ANN are different for each of the two systems. The data classification in FDA system can be correlated with:

a. initial level (10s),
b. level during submersion (75s),
c. peak level of 100s - correlated with drop appearance,
d. three levels for 125, 150, 200s of measuring cycle time - correlated with drop forming,
e. final signal level.

We used a 4 layers perceptron network, with 6 and 5 nodes in hidden layers and sigmoid transfer function. This network giving with training the RMS error of 0.017 and the correlation coefficient of 0.998. These values and the ANN output results were satisfactory, Table 6.

No. of ANN output	Std	Correlation
1	0.01770	0.99906
2	0.02971	0.99713
3	0.02990	0.99730
4	0.02087	0.99871
5	0.02413	0.99811

Table 6. The outputs parameters of trained ANN for FDA water classification

The data classification in the capillary system can be correlated with:

a. initial level,
b. slope before bubble creation, (5s),
c. time of bubble creation,
d. time of bubble absorption, or 0 when bubble shoot the liquid,
e. final signal level.

In this case we also used a 4 layers perceptron network giving with training the RMS error of 0.12 and the correlation coefficient of 0.89. The ANN output test data are presented in Table 7.

No. of ANN output	Std	Correlation
1	0.17347	0.90426
2	0.06646	0.98702
3	0.05557	0.98936
4	0.22671	0.83018
5	0.27209	0.74438

Table 7. The outputs parameters of trained ANN for capillary water classification

3. Conclusion

We have shown that intelligent photonic sensors are capable of classifying wastewater parameters and can be easy in operation. The proposed sensors work in contact with the examined liquid. The proposed construction is based on new sensing ideas. The classification of grey wastewater treatment, water with surfactant 5ml/l solution and clear water was performed satisfactorily in both systems. The FDA system was superior to the capillary system in terms of classification parameters of wastewater, but the capillary system is simpler in construction and does not require moving parts. The capillary system does not require head cleaning; the capillary optrode is disposable, which is an advantage. The cost of the examination is relatively small. Before the systems can be used for practical applications further system integration and automation of the measurement process are required.

4. Acknowledgements

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Analysis and Selective Treatment of Radioactive Waste Waters and Sludges

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1. Introduction

In the Hungarian PWR-type nuclear power plant Paks (four 500 MW_e capacity VVER-440/213 blocks) the radioactive waste waters are collected in common tanks. These water streams contain radioactive isotopes in ultra-low concentration and inactive compounds as major components (borate 1.7 g/dm^3, sodium-nitrate 0.4 g/dm^3, sodium-hydroxide 0.16 g/dm^3, and oxalate 0.25 g/dm^3).

Up to the present the low salinity solutions were evaporated (by adding sodium-hydroxide) till 400 g/dm^3 salt content (**pH~13**) and after solidification by cementing buried. There is about 6000 m^3 concentrated evaporator bottom residue in the tanks of the PWR. In order to separate the inactive salt content before cementing a Liquid Wastewater Treatment Technology (LWT see Figure 1.) was developed to treat this wastewater before solidification and burial (Pátzay et al., 2006).

The long-life radionuclides are present in very low concentration (10^{-9}-10^{-12} mol/dm^3) as ions, suspended, colloid particles and in complex (EDTA, oxalate, citrate) form. In this technology the SELION CsTreat cesium selective ion exchanger is used for the selectice separation of radiocesium isotopes (^{134}Cs, ^{137}Cs). The SELION **CsTreat** cyanoferrate based cesium-selective ion exchanger is not stable at pH>11 (see reaction equation below), so the use of CsTreat needs partial neutralisation of the evaporator bottom residue to pH~9-11, and during neutralisation sodium-borate crystals precipitate with about 15-30% of the radioactivity.

$$K_2Co[Fe(CN)_6] + 2OH^- \Rightarrow 2K^+ + [Fe(CN)_6]^{4-} + Co(OH)_2 \qquad (1)$$

The contaminated crystals should be washed to remove the radioactive isotopes from the crystals. To eliminate the generation of radioactive borate crystals and additional wastes we have developed a $M_2Ni[Fe(CN)_6]$ type cesium selective granulated ion exchanger (where M is an alkali ion) which has good stability even at pH>11.

Based on this new cesium selective ion exchanger stable at pH>11 we have modified the radioactive evaporator bottom residue treatment technology at the nuclear power plant. The basic idea of the new technological scheme is the selective separation of all radionuclides with inorganic sorbent materials or reagents in very simple processes without any prior neutralization, dilution. After the separation of all radionuclides the inorganic salt content

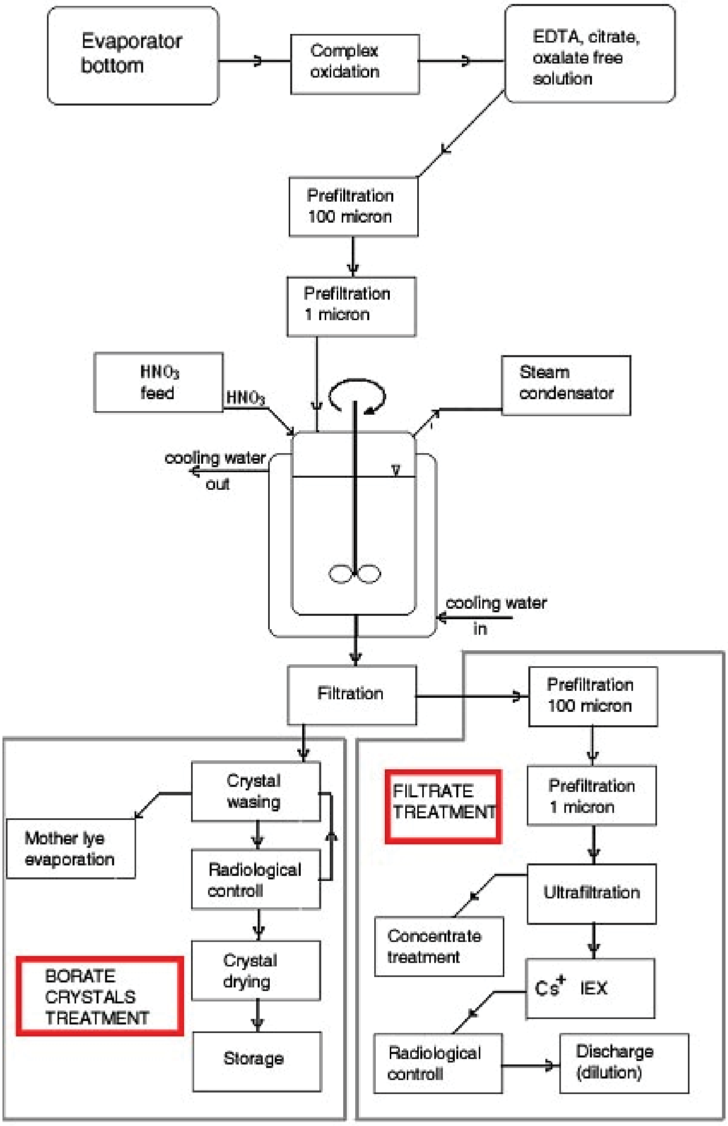

Fig. 1. *The Liquid Wastewater Treatment Technology*

(borates, partially nitrates) could be separated with crystallization using nitric acid neutralization and the inactive crystals could be treated as chemical waste. In the first part of this report this modified separation technology will be discussed.

In the Nuclear Power Plant Paks at the bottom of some radioactive liquid waste containing tanks there are segregated sludge phases, containing more or less organic complex builder compounds (including EDTA, citrate and oxalate compounds). The radioactive waste water treatment technology, developed at the plant is not suitable to treat sludges, so a modified technology is needed using cementing as solidification. For this technology the detailed analysis of these sludge phases are of great importance. According to this problems we started a research work to investigate the international experience in the analysis of radioactive sludges and fulfilled laboratory scale experiments for chemical and radiochemical analysis of different sludge samples. In the second part of this report the analysis of these radioactive sludges will be discussed.

2. The modified liquid wastewater treatment technology

The developed modified technology consists of the following parts:

- Firstly the high salt content, strongly alkaline (*pH*~13-14) evaporator bottom residue is **microfiltered**.
- Then the free EDTA, citrate, oxalate content is **oxidized** with underwater plasma torch and with Fenton oxidation (in this process Co isotopes removed by precipitation as oxide-hydroxide and can be separated by filtration). The treated solution is microfiltered and ultrafiltered.
- Selective separation of the radioactive cesium isotopes (^{137}Cs, ^{134}Cs) using ion exchange material stable at alkaline pH.
- Crystallization of borates from the mother lye by neutralization with nitric acid.

The modified waste treatment technology was tested at the NPP. After microfiltration about 500 dm^3 evaporator bottom residue was oxidized with underwater plasma torch for the EDTA, citrate and oxalate removal. The oxidized evaporator bottom residue was then microfiltered and ultrafiltered to remove suspended matter and cobalt precipitation from the solution having a pH~12.3 The separation efficiency of the ultrafiltration is shown in Table 1.

	^{60}Co activity concentration (Bq/kg)	%	^{134}Cs activity concentration (Bq/kg)	%	^{137}Cs activity concentration (Bq/kg)	%
Feed	2310	100	1350	100	181000	100
Permeate	258	11.2	1210	89.6	164000	90.6

Table 1. *Ultrafiltration of the waste water after oxidation of the complex compounds*

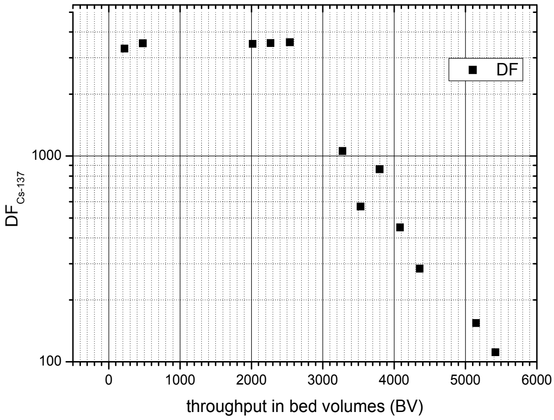

Fig. 2. *Breakthrough curve of ^{137}Cs (BV-bed volume)*

The solution purified from radioactíve cesium was then acidified with concentrated nitric acid in 20 dm^3 batches in a cooled mixed reactor till pH~9.0. The crystallization reactor is shown in Figure 3.

Fig. 3. *The crystallyzation reactor*

Fig. 4. *The separated wet crystals by the original (left) and by the modified (right) technology*

The crystals were separated by filtration, dried at 50 ^{0}C and weighted. The crystalline product contained mainly sodium-metaborate ($NaBO_2*8H_2O$). Heating the product above 55 ^{o}C the crystalline phase released four water molecules and $NaBO_2*4H_2O$ formed. Figure 4 shows the separated wet crystals by the original and by the modified technology.

The measured specific radioactivity of the separated, dried crystalls and the unconditional clearance limit values are summarized in Table 2.

Radionuclide	Measured specific activity (Bq/g)	Unconditional clearance limit (Bq/g)
^{51}Cr	1.42E-02	30
^{54}Mn	1.19E-03	1
^{58}Co	1.01E-03	1
^{59}Fe	1.93E-03	0.9
^{60}Co	1.17E-03	0.9
^{65}Zn	2.66E-03	2
^{95}Nb	1.10E-03	0.9
^{95}Zr	1.81E-03	3
^{106}Ru	1.15E-02	1
^{110m}Ag	1.83E-03	0.9
^{124}Sb	1.83E-03	0.9
^{125}Sb	7.63E-03	1
^{134}Cs	1.66E-03	0.9
^{137}Cs	1.11E-01	2
^{144}Ce	1.02E-02	30
^{154}Eu	2.59E-02	0.9
^{3}H	2.94E-02	2000
^{14}C	1.91E-05	200
^{55}Fe	3.01E-05	100
^{59}Ni	6.20E-06	800
^{63}Ni	2.72E-04	300
^{90}Sr	3.19E-02	1
^{99}Tc	7.19E-05	1
^{129}I	1.24E-09	0.9
^{234}U	4.69E-07	0.9
^{235}U	1.71E-07	0.9
^{238}U	1.09E-07	0.9
^{238}Pu	4.83E-07	0.9
^{239,240}Pu	3.62E-07	0.9
^{241}Am	5.48E-08	0.9
^{242}Cm	4.01E-07	0.9
^{244}Cm	4.26E-07	0.9

Table 2. *The measured specific radioactivity of the separated, dried crystalls and the unconditional clearance limit values*

Based on our modification of the original wastewater treatment technology in the Hungarian Nuclear Power Plant we get beneficial results summarized as follows:

- The use of the new cesium selective ion exchanger eliminates the acidification of the evaporator bottom residue before the cesium removal by ion exchange.
- Hence we can avoid the formation of borate crystals contaminated with radionuclides of cesium etc. and the additional washing of the separated crystals for the radioactivity removal.
- According to measured specific activity data we are able to release the dried solid crystals from the NPP and could be used as non-radioactive borate chemical.

3. Chemical and radiochemical analysis of radioactive sludges fron NPP Paks

According to the international experiences the sampling process depends on the sludge characteristics. The first step of the sampling process is a previous sampling to determine the boundary between the supernatant and sludge layers. This is followed after 3-4 days by the sampling. For diluted, liquid type sludges below the supernatant layer we can detect very often a crystalline salt and amorf sludge layer too. Sampling are usually done from the top, intermediate and bottom layers using a sampling pipe and vacuum For the concentrated sludges the samples are taken from different layers of the sludge phase.

Following the sampling the sludge samples are photographed and characterized. The samples for organic content determination (TC, TOC, TIC) are collected in glass bottles, the samples for ion chromatographic analysis are stored in polyethylene botles at 4 ^{0}C. The liquid samples are analysed for pH.

We investigated two times three sludge samples taken from the tanks 02TW30B001, 02TW01B001, 01XZ06B001 of the Paks NPP. The sample characteristics are summerized in Table 3.

Sample Code	Tank code	Sample type	Sampling time
P3	02TW30B001	sludge from the evaporator, pH~13	2008. 11. 06. 11:45
P4	02TW01B001	settled sludge from diluted waste water tank, pH~8	2008. 11. 06. 11.45
P5	01XZ06B001	sludge from the wash-house waste	2008. 11. 07. 10.30
P3-2	02TW30B001	sludge from the evaporator, pH~13	2009.01.20.
P4-2	02TW01B001	settled sludge from diluted waste water tank, pH~8	2009.01.20.
P5-2	01XZ06B001	sludge from the wash-house waste	2009.01.20.

Table 3. Sludge sample characteristics

The samples P3 and P4 are seen on Picture 1. The P3 and P4 samples contained liquid phase too, while sample P5 contained only solid, consistent type phase.

Picture 1. The samples P3 and P4 shaked(left) and settled(right)

Physical chracateristics of the sludge samples

Because of the high dissolved and suspended content, high pH and other characteristics the direct determination of the sludge densities, the total solid content (TS), the dissolved and undissolved solid content (DS, UDS) was questionable. Separation of the liquid content of the composite sludge samples by vacuum filtration resulted a given amount of liquid phase remaining between the particles of the sludge sample and the determination of the total solid content of the sample is also problematic. Washing the dried sample may cause some dissolution losses. Because the above mentioned reasons we used an indirect method suggested by analysts (Ceo et al.,1990) for the determination of the densities and solid content of the samples. The results are shown in Table 4.

	original sludge				**centrifuged sludge**			
Sample	DS*	liquid mass	UDS**	flocculation density	DS*	liquid mass	UDS**	flocculation density
	%	g/ml	%	g/ml	%	g/g	%	g/ml
P3-2	20.417	0.971	10.174	1.139	15.022	0.847	24.415	1.179
P4-2	0.121	0.818	18.274	1.208	0.065	0.772	22.851	1.355
P5-2	-	-	-	-	-	-	-	-

(* DS-dissolved solid content, **UDS-undissolved solid content, floculation density-undissolved solid density)

Table 4. Physical charactersitsics of the sludge samples P3-2, P4-2 and P5-2

Organic content of the samples

We determined the TOC, TC and COD values of the centrifuged at 4000g supernatant portion of the samples P3-2 and P4-2, characterizing the dissolved organic content of the sludge samples. For the TC and TOC determination we used a SHIMADZU OceaniaTOC-V CS device, and COD was determined by the potassium bicromate method using potassium-hydrogen-phtalate reference. Results are seen in Table 5.

Sample	dilution	COD	TC	TIC	TOC
		mg/l	mg/l	mg/l	mg/l
P3-2	100	3785	5271	4177	1094
P4-2	100	971.8	250.5	140.1	110.3

Table 5. Organic content of the centrifuged supernatants

It is seen that sample P3-2 contains higher organic content then sample P4-2.

Chemical composition

Samples P3-2 and P4-2 contained supernatant liquid, which was separated by centrifuging at 4000g for 15 minutes and the separated liquid phase was analysed for anions (fluoride, chloride, nitrate, phosphate and sulfate) and for cations (lithium, sodium, potassium, ammonium, calcium and magnesium) ,by ion chromatography with dilution factors between 100 and 1000. We used an IC 861 Metrohm type ionchromatograph with a conductometric detector using an *Asupp4-250* type anion, and a *C3-250 type cation exchanger columns, with a Metrohm 837* type degasser and a *Metrohm 838* type sampler.

Alkalinity (hydroxide, carbonate and hydrogen-carbonate) was determined by titrimetry. Result of the supernatant chemical analysis of samples P3-2 and P4-2 are shown in Table 6.

P3-2					
Cations			**Anions**		
	mg/l	mekv/l		mg/l	mekv/l
Na	70389.60	3354.9	F	46.27	2.67
NH_4	0	0	Cl	346.16	10.69
K	4021.67	112.69	NO_3	27094.24	478.80
Mg	0	0	PO_4	199.77	6.92
Ca	0	0	SO_4	1170.01	26.69
Mn	0	0	OH	20199.16	1301.19
			CO_3	44809.96	1636.66
Sum	74411.27	3467.59	Sum	93865.58	3463.64
P4-2					
Cations			**Anions**		
	mg/l	mekv/l		mg/l	mekv/l
Na	311.2	13.53	F	0	0
NH_4	0	0	Cl	8	0.22
K	0	0	NO_3	40	0.64
Mg	0	0	PO_4	12.2	0.38
Ca	0	0	SO_4	3.7	0.08
Mn	0	0	OH	0	0
			CO_3	263.67	8.79
			HCO_3	289.70	4.75
Sum	311.2	13.54	Sum	617.27	14.87

Table 6. Chemical analysis of the centrifuged supernatants of samples P3-2 and P4-2

It is seen from the ionic composition of the supernatants, that in the P3-2 sample sudium is the main cation and important anions are carbonate, hydrogencarbonate and hydroxide. P4-2 supernatant sample contains only small amount of sodium and hydrogencarbonate ions.

The chemical composition of the remaining after centrifugation solid phases and of the sample P5-2 was determined by simultaneous wasing water analysis and fusion of the solid phases using potassium-hydroxide fusion and hydrochloric acid dissolution and sodium-peroxyde-sodium-hdroxide fusion and hydrochlorid acid dissolution. Washing was completed by washing 1 g dry sample with 10 ml ultrapure water at 25 ^{0}C and 350 rpm stirring for 10 minutes, then filtered with a 0,45 micrometer size microfilter. Filtrates were analsed with ion chromatography. According to results of analysis based on five paralell measurements sodium cation and chloride, hydroxide, nitrate, phosphate and hydrogencarbonate anions are present in the washing water samples.

Chemical composition of the solid phase sludges were also determined by the fusion of the solid phases using potassium-hydroxide fusion and hydrochloric acid dissolution and sodium-peroxyde-sodium-hdroxide fusion and hydrochlorid acid dissolution.

Fusion using potassium hydroxide was completed with ~1g dry sludge mixed with 5 g potassium-hydroxide and heated for 30 minutes and after cooling dissolved in 50 ml conc.

HCl and 50 ml ultrapure water. The determined potassium content was recalibrated using the potassium content of washing water samples, supposing that all potassium content is soluble in water. The total ionic content of the sludge samples P3-2 and P4-2 was calculated by summing the ionic contrations determined by wasing water and fusion solution analysis. The equivalent concentrations of the anions were calculated according to the distribution af the anions in the centrifuged supernatants of samples P3-2 and P4-2 and to the distributions of the anions in the washing water of sample P5-2.

The variances of the five repeated analysis results of this fusion was between 0,4-39% for the different ionic components.

For each sludge sample we completed also a sodium-peroxide-sodium-hydroxide fusion and a hydrochloric acid dissolution too. In the fusion process we mixed ~0.25 g dry sludge with a mixture of 1.5 g sodium-peroxide and 1 g sodium-hydroxide and heated for 15 minutes at 600 ^{0}C. After cooling the residue was dissolved with a mixture of 50 ml cc. HCl and 50 ml ultrapure water. Solutions were analyzed by ion chromatography. The variances of the five repeated analysis results of this fusion was significantly higher (23-87%) then in case of the potassium.hydroxide based fusion, so we used the results of the smaller variances.

Results are summarized in Table 7.

a) P3-2

Cations					
	KOH fusion+HCL			total (supernatant+fusion)	
	mg/g dry sludge	mg/l sludgep	mekv/l sludge	mekv/l sludge	mg/l sludge
Na	200.59	17016.05	435.17	3790.07	87132.97
NH_4		0	0	0	0
K	3.83	324.89	8.31	121.00	4731.58
Mg	18.82	1596.50	131.37	131.37	1596.50
Ca	62.42	5295.08	264.22	264.22	5295.09
Mn	281.28	23860.98	1963.46	1963.46	53934.36
Sum	285.66	24232.54	839.07	4306.67	98756.14
Anions total equivalent capacity ditributed according to supernatant distribution					
F	-	12.34	0.64	3.31	63.07
Cl	-	92.31	2.60	13.30	471.59
NO_3	-	7225.88	116.53	595.33	36913.83
PO_4	-	53.29	1.68	8.60	272.26
SO_4	-	312.03	6.49	33.18	1594.07
OH	-	5383.97	316.70	1617.90	27504.34
CO_3	-	11950.68	398.35	2035.02	61050.69
Sum	-	25030.54	843.03	4306.675	127869.90

b) P4-2

Cations					
	KOH fusion+HCl			total (supernatant+fusion)	
	mg/g dry sludge	mg/l sludge	mekv/l sludge	mekv/l sludge	mg/lsludge
Na	39.04	4199.53	182.67	196.20	4510.73
NH_4	0	0	0	0	0
K	0	0	0	0	0
Mg	11.12	1196.18	98.43	98.43	1196.18
Ca	68.49	7367.47	367.64	367.64	7367.47
Mn	136.66	14700.52	267.58	267.58	7350.26
Sum	118.65	12763.18	648.74	662.27	13074.38
Anions total equivalent capacity ditributed according to supernatant distribution					
F	-	0	0	0	0
Cl	-	348.30	9.82	10.05	356.30
NO_3	-	1741.50	28.08	28.73	1781.50
PO_4	-	531.16	16.78	17.16	543.36
SO_4	-	161.09	3.35	3.43	164.79
OH	-	0	0	0	0
CO_3	-	11479.55	382.65	391.44	11743.22
HCO_3	-	12612.84	206.71	211.46	12902.54

c) P5-2

Cations				
	KOH fusion+HCl			Total(fusion)
	mg/g dry sludge	mg/l sludge	mekv/l sludge	mekv/l sludge
Na	29.29	11704.87	509.13	509.13
NH_4	0	0	0	0
K	0	0	0	0
Mg	6.967	2784.153	229.10	229.10
Ca	60.99	24372.82	1216.21	1216.21
Mn	138.75	55447.28	1009.27	1009.27
Sum	97.247	38861.85	1954.44	1954.44
Anions total equivalent capacity ditributed according to washing water distribution				
F	-	0	0	0
Cl	-	1535.89	43.32	43.32
NO_3	-	1000.38	16.13	16.13
PO_4	-	21549.04	680.70	680.70
SO_4	-	3731.21	77.69	77.69
OH	-	0	0	0
CO_3	-	0	0	0
HCO_3	-	69352.12	1136.60	1136.60
Sum	-	97168.64	1954.44	1954.44

Table 7. Ionic composition of solid sludges using KOH fusion and the total sludge composition

We determined also the the undissolved oxalate content of the sludges by treating the samples first with cc. HCl for the dissolution of iron complexes, followed with a cc. nitric acid dissolution. The undissolved oxalate content of the samples P3-2, P4-2 and P5-2 was 4.177, 6.848, 23.362 meq/l accordingly.

The EDTA content of the centrifuged supernatants were determined with ion chromatography with the method suggested by analysts (Krokidis et al.,2005). The EDTA content (as Na_2H_2-EDTA*$2H_2O$) was for centrifuged supernatant samples P3-2 and P4-2 10.86 and 12.82 mmol/l accordingly. The iron and manganese content of the sludge samples were determined by AAS.

The borate content of the centrifuged supernate samples P3-2 and P4-2 was determined also with ion chromatography with the method suggested by analysts (Tapparo et. al.,1998). The borate content was for centrifuged supernatant samples P3-2 and P4-2 164.35 and 1.172 g/l accordingly.

The sludge composition was then calculated based on the ion chromatographic and titrimetric analysis of the supernatant, washing water, fusion samples and on the ICP-MS analysis. The calculated sludge compositions are summerized in Table 8.

a) P3-2

Composition	mekv/l sludge	mmol/l sludge	mg/l sludge
NaOH	1618	1618	63084.53
Na_2CO_3	2035	1017.5	107843.6
$NaNO_3$	137	137	11644.27
KNO_3	121	121	16723.8
$Ca(NO)_3)_2$	264	132	13211.77
$MgCO_3$	125	62.5	2634.819
sum of ionic	4300	3088	215142.8
$Fe(OH)_3$	2279.7	759.9	81209
$Mn(OH)_2$	109.7	54.85	4879.5

b) P4-2

Composition	mekv/l sludge	mmol/l sludge	mg/l sludge
$NaNO_3$	28.73	28.73	2441.898
Na_2CO_3	163.47	81.735	8662.995
$CaCO_3$	227.93	113.965	11406.67
$Ca(HCO_3)_2$	139.71	69.855	11324.5
$Mg(HCO_3)_2$	71.79	35.895	5252.853
$MgCl_2$	10.05	5.025	478.4353
$Mg_3(PO_4)_2$	17.16	2.86	751.7733
$MgSO_4$	3.43	1.715	206.4219
Sum of ionic	662.27	339.78	40525.54
$Fe(OH)_3$	6134.34	2044.78	218523.6
$Mn(OH)_2$	581.18	290.59	25848.81

c) P5-2

Composition	mekv/l sludge	mol/l sludge	mg/l sludge
NaCl	43.32	43.32	2531.742
$NaNO_3$	16.13	16.13	1370.965
Na_3PO_4	449.68	149.8933	24573.63
$Ca_3(PO_4)_2$	231	38.5	11942.04
$Ca(HCO_3)_2$	985.21	492.605	79858.36
$Mg(HCO_3)_2$	151.4	75.7	11077.89
$MgSO_4$	77.71	38.855	4676.689
sum of ionic	1954.45	855.0033	136031.3
$Fe(OH)_3$	4572.75	1524.25	162895.3
$Mn(OH)_2$	12.742	6.371	566.73

Table 8. The calculated composition of the sludge samples

Radiochemical composition

The radiochemical composition of the sludge samples was determined using gamma- and alpha-spektrometry and liquid scintillation measurements. For the gamma-spektrometry we used an ORTEC Model GMX25P4-76-C, Gamma-X HPGe Coaxial Detector with a CarbonFiber window, connected to a multichannel anlyser ORTEC DSPEC-jr-2.0, the spectra were measured with 100 cm^3 poliethylene sample with a 3600 sec counting time. Spectra were evaluated with a Gamma Vision – 32 code. The results show, that in the P3-2 sludge ~99,5% of the gamma-activity is from ^{137}Cs, ^{134}Cs and ^{60}Co isotopes. The centrifuged supernatant contains 92-92,7% of the cesium, 14,26% of the cobalt and 91,35% of the total gamma-activity. In the P4-2 sludge ~99,4% of the gamma-activity is from ^{60}Co and ^{137}Cs, ^{134}Cs ^{54}Mn isotopes. The centrifuged supernatant contains only 0.62% of the total gamma-activity. After fusion of the solid parts of the sludges for the P3-2 sample 97% of the gamma activity is due to ^{137}Cs and ^{60}Co, while for the P4-2 sample 89% of the gamma-activity is due to ^{60}Co, 5,7% ^{137}Cs and there is also ^{54}Mn isotope present. P5-2 sample contains less activity of which 50,6% is ^{60}Co and conatins ^{54}Mn and ^{137}Cs isotopes too.

Finally we determined the alpha- and beta-emitting isotopes in the samples too using preconcentration and salt removal techniques.For the alpha-activity measurements we used TRU columns and alpha-spectrometry and for the determination of beta-activity liquid scintillation technique. Detailed description of these measurements will be submitted later. Resultst for the KOH fusion samples radionuclide analysis are shown in Table 9.

Isotope	Sample P-3-2		Isotope	Sample P-4-2		Isotope	Sample P-5-2	
	Act. conc. Bq/kg	σ Bq/kg		Act. conc. Bq/kg	σ Bq/kg		Act. conc. Bq/kg	σ Bq/kg
^{234}U			^{234}U			^{234}U		
^{238}U			^{238}U			^{238}U		
^{239,240}Pu	78.5	±8.68	^{239,240}Pu	549	±21.1	^{239,240}Pu	127	±12.7
^{238}Pu	78.1	±8.79	^{238}Pu	429	±18.5	^{238}Pu	194	±15.7
^{241}Am	73.8	±3.78	^{241}Am	771	±16.5	^{241}Am	99.8	±8.82
^{244}Cm	11.9	±1.47	^{244}Cm	124	±5.64	^{244}Cm	54.5	±5.68
^{90}Sr	21500	±1080	^{90}Sr	10200	512	^{90}Sr	20700	±1040

Table 9. The measured alpha- and beta-activities of the fused samples with KOH

4. Conclusion

The evaporator bottom residue in the NPP Paks contains high inactive salt content (~400g/dm^3) with trace amount of radioactive fission and activation products. The separation of the bulk borate and nitrate from the radioactive minor components is of great importance before solidification. The modified separation technology produces clean crystals without radioactive pollutants above the „unconditional" clearance limits. The final volume reduction factor is higher then 1000.

There are some tanks at the power plant containing sludge type radioactive waste containing more or less liquid phase too. The general physical and chemical charactersitics (density, pH, total solid, dissolved solid etc.) and chemical and radiochemical composition of these sludges are important information for volume reduction and solidification treatment of theese wastes. Based on the literature sources we have investigated and constructed a complex analysis system for the radioactive sludge and supernatant analysis, including the physical, as well as the chemical and radiochemical analysis methods. Using well known analysis techniques as ion chromatography, ICP-MS, AAS, gamma-and alfa-spectrometry and chemical alkaline fusion digestion and acidic dissolution methods we could analyze the main inorganic, organic and radioactive components of the sludges and supernatants. Determination of the mass and charge balance for the sludge samples were more difficult then for the supernantant samples. Not only are there assumptions required about the chemical form and the oxidation state of the species present in the sludge, but many of the compounds in the sludge are mixed oxides which are not directly measured. Also, the sludge is actually a slurry with a high water content. The interstitial liquid is in close contact with the sludge, and there are many ionic solubility equilibriums. The anion data for the sludge samples are based on the water soluble anions that would be available to a water wash. The water wash would not account for the insoluble hydroxides, carbonates, and mixed oxides present. The insoluble species do not contribute to the charge balance, and the cation charge is not used in the calculation. Most of the nitrate reported for the sludge is due to the interstitial liquid. Considering the limitations of these calculations, the mass balance was within the analytical error (±20%) for the sludge samples. There were three sample preparation methods used to investigate the total anion content of the sludge samples, which included water leach, potassium-hydroxide and/or sodium peroxide/sodium hydroxide fusion and acidic dissolution.

5. References

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Part 2

Evaluation of Waste Water Effects on the Environment

10

Effects of Waste Water on Freshwaters in Semiarid Regions

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1. Introduction

Some freshwater ecosystems have received waste water for many centuries (Alvarez-Cobelas & Verdugo, 1995), *i.e.* the Latium wetlands close to Rome or the Thames and the Spree rivers downstream London and Berlin, respectively. The strong development experienced in many areas of the world in the 20th century has resulted in increasing waste water disposal almost everywhere that has increasingly threatened freshwater ecosystems receiving these loadings. The concern of human health and, later, ecosystem health resulted in the implementation of waste water treatment facilities in many developed countries, such as those of Northern and Central Europe, USA, Canada and Japan (Tchobanoglous et al., 2003), which diminished wastewater inputs to freshwater environments. Unfortunately, this has not been the case in many semiarid countries whose economy or, more often, some lack of concern for environmental quality does not enable funding enough for these otherwise expensive facilities. While waste water pollution cannot always be abated, its effects are likely to be diminished if enough water is available to produce both dilution and wash out, as often occurs in cold temperate and tropical environments. Unfortunately, this is not the case in semiarid areas where rainfall is unevenly distributed throughout the year, also showing a strong interannual variability. For example, Fig. 1 depicts the long-term (1945-2006) annual precipitation falling on a semiarid central Spanish area, which results in an average of 418 ± 128 mm/year, ranging 189-857 mm/year.

In fact, traditional approaches to water management in semiarid regions have been based more on the increase of water availability rather than improving the water quality of waste waters to make them feasible for future use. In water shortage scenarios, domestic lifestyle adaptations and optimization of water consumption by both agriculture and industry have been managed to maintain the balance between water supply and demand. However, although this balance could be achieved and the amount of waste water reduced, the characteristic low water flow of semiarid rivers makes impact of waste water discharge in freshwater ecosystems stronger. Streamwater discharge to wetlands and lakes is highly variable over time in semiarid areas. Fig. 2 shows an example of these fluctuating water flows of a semiarid river that drains to a central Spanish wetland (Las Tablas de Daimiel National Park). Semiarid regions of the world are confronted with a largely unpredictable

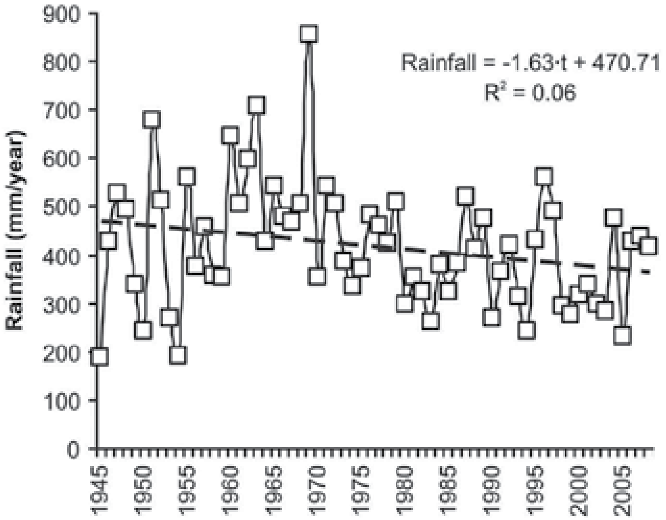

Fig. 1. Long-term series of rainfall in the vicinity of Las Tablas de Daimiel National Park, a freshwater marshland in Central Spain. Data were compiled by the Spanish Meteorological Institute. The long-term trend (P < 0.05) is also shown.

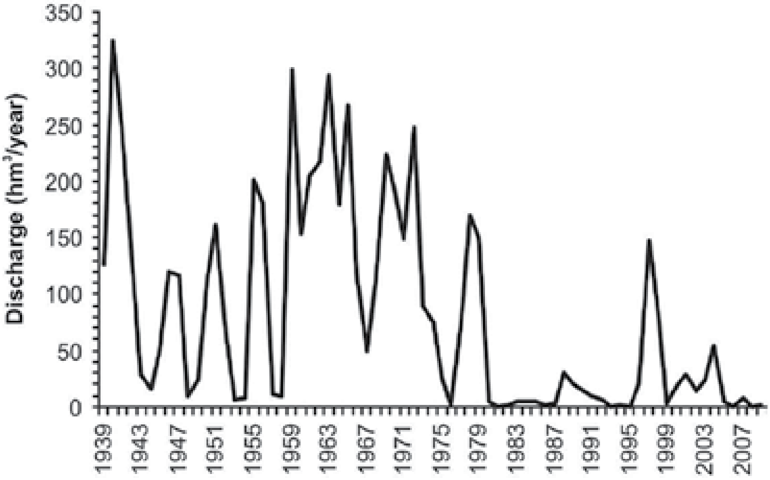

Fig. 2. Long-term water discharge of Gigüela river in central Spain, draining to Las Tablas de Daimiel National Park. In addition to natural changes, there has been a stronger variability from the seventies onwards as a result of groundwater exhaustion that diminished river flow. Data have been gathered by the Guadiana Water Authority, which is the Spanish administrative office dealing with water quantity and quality in the area.

climate, often recognizing water availability as the single most important limiting resource for the conservation of aquatic ecosystems. Besides, the natural fluctuating hydrology is often increased by anthropogenic variability arising from water abstraction for irrigation purposes. This impact is certainly more frequent in semiarid areas whose agriculture heavily relies upon water resources that can be either stored in aquifers or flowing in streams, thus diminishing the amount of water available for diluting pollutants and cleaning freshwater environments. That is, the high variability of water availability very often experiences a positive feedback as a result of unsustainable agricultural consumption (Postel, 1992), *i.e.* unsustainable irrigation promotes more variability of water availability. Furthermore, this excessive irrigation usually uses groundwater as the main water source, which it is often the single source on which most semiarid ecosystems depend. Already in many semiarid regions aquifer drawdown by irrigation pumping is such that aquifers appear overexploited. Therefore, it is not unusual that semiarid aquatic ecosystems receive sewage as the almost exclusive inflow, becoming the main threat for conservation purposes. These reduced, natural and man-made inputs of water to aquatic environments usually fail to minimize pollution impacts triggered by waste water. The point of view that water is water regardless of its quality results in overlooking the basis of water management in these areas.

Under these scenarios of water management, it is obvious that the information available is rather scarce and, more specifically, hardly addresses the topic of waste water effects on freshwaters in semiarid regions. The conservation of these valuable freshwater ecosystems demands the need to consider water quantity and quality jointly in any water policy.

Ecological effects of waste water on ecosystems located downstream have been the core of much research after the initial studies by Kolkwitz & Marsson (1908) and Streeter & Phelps (1925). Unfortunately, there are pitfalls in this approach. First, it is not of a widespread nature, mostly pertaining to cold temperate areas where water availability is rarely limited. Second, effects are only sought in the changing of biological communities and their species numbers, paying no attention to other processes such as biogeochemical effects, biomass and productivity effects, and food web effects.

The main goal of this chapter is to outline how waste water (either raw or treated) discharges can affect the ecological performance of semiarid freshwaters downstream. We will review the water quality of waste water and later consider both abiotic and biotic effects of those waters on these ecosystems; since there are very few contributions on this topic, we will mainly rely on our own research, mostly reporting unpublished information. We will also suggest some easy-to-use remedial actions to cope with these environmental impacts posed by waste water. To conclude, we will describe some ideas on future research on the topic.

2. Methods

This chapter mostly relies on data of our own because there is not much published evidence on the effects of waste water on the ecological performance of semiarid freshwater ecosystems. Therefore, we will report the studies available on the topic, which are not many. Since we have been working over more than 30 years on these impacts in some Spanish ecosystems, including rivers and wetlands and waste water treatment facilities as well, we will also report unpublished data. Chemical oxygen demand, total nitrogen and total phosphorus contents have frequently been measured following APHA (1989) procedures. Also data compiled by some Spanish offices, such as the Guadiana Water Authority and the National Meteorological Institute, will be used to describe raw and treated waste water quality. Anyway, we will mainly focus on phosphorus because it is often the main factor limiting productivity in lakes (Lewis & Wurtsbaugh, 2008).

We have also undertaken chemical and biological measurements in streams, lakes and wetlands over the years. Elemental composition of sediments has also been measured using a CHN Perkin-Elmer analyzer. Species richness of algae, macrophytes and macroinvertebrates have also been recorded in streams and wetlands. Cover, biomass and productivity measurements of phytoplankton, submerged-, emergent- and pleustonic vegetation have also been carried out (see Alvarez-Cobelas et al., 2011, and Alvarez-Cobelas & Cirujano, 1996, for an overview of methods).

3. Wastewater quality

Water supply and treatment often received more priority than wastewater collection and treatment. The trend in human population increase, however, might result in greater emphasis on wastewater treatment. Although there is a growing awareness of the impact of sewage contamination on rivers and lakes, few countries recognize that it may affect valuable ecosystems severely because waste water is deemed for managers and politicians to be ashamed of. Hence, it is not surprising that there are too few studies reporting its effects on valuable ecosystems downstream. Table 1 shows some stagnant water bodies that receive waste water in semiarid areas. It is sure that there will be many more because treatment facilities are less common in these countries than in higher developed countries and because the maintenance of operations, and hence the improvement of water quality of the treated effluents to be discharged to freshwaters, is much better in these countries than in semiarid, mostly poorer countries.

NAME	COUNTRY	LATITUDE	LONGITUDE
Chott Aïn el Beïda (playa lake)	Algeria	32° N	5° E
Nature Park Krpacki rit (floodplain wetland)	Croatia	46° N	19° E
Lake Vistonis, Porto Lagos and Lake Ismanis	Greece	41° N	25° E
Lakes Volvi and Koronia	Greece	40° N	23° E
Cagliari pond	Italy	39° N	9° E
Punte Alberete wetland	Italy	45° N	11° E
Babícora lagoon	Mexico	29° N	108° W
Souss Massa wetlands	Morocco	30° N	10°W
Albufera de Valencia lagoon	Spain	39° N	0° W
Alcázar de San Juan lagoons	Spain	39° N	3° W
Doñana National Park (wetlands and marshes)	Spain	37° N	6° W
El Hondo wetland	Spain	38° N	1° W
Manjavacas lagoon	Spain	39° N	3° W
Las Tablas de Daimiel National Park (freshwater marsh)	Spain	39° N	4° W
Lake Burdur	Turkey	38° N	30° E

Table 1. Some stagnant freshwater ecosystems in semiarid areas which experience wastewater pollution. Most data are either reported in www.ramsar.org or are authors' unpublished data.

One feature of either raw or treated waste water in semiarid areas that deserves mention is the high variability of its water quality indices (Table 2). Besides, in comparison to most temperate countries, domestic wastewater in arid areas like the Middle East are up to five times more concentrated in the amount of chemical oxygen demand per volume of sewage because the domestic water consumption is lower (Al-Salem, 1987). This is extremely high and may cause a large amount of sludge production, high-energy consumption for aeration, operational problems, and high consumption of polymers and clean water for drying the sludge after digestion (Massoud et al., 2009). Could this mean that conventional Western treatment systems may even be technologically inadequate to handle the produced sewage in semiarid regions? Traditional treatment systems are implemented without considering the appropriatedness of the technology for the economy, culture, land, and climate. If the aridity of climate tends to increase the concentration of pollutants in waste waters because water use by the human population living in these regions is often rather low, then the implanted treatment systems must address this peculiarity; otherwise chances of ecologically successful treatment are very limited. Although it is not the purpose of this chapter, there is clear evidence that the application of conventional treatment systems in semiarid countries cause several problems in the waste water plant functioning, revealing its inability to mitigate the adverse effects to freshwater ecosystems. Coupled with this, probably the lack of environmental control mechanisms, the absence of long-term environmental planning and the weakness of the legal requirements are preventing to achieve the necessary improvements to solve the problem of waste water discharges in these regions.

		Alcázar de San Juan	Typical domestic wastewater
Biochemical oxygen demand	(mg O_2/L)	25-1750	100-500
Chemical oxygen demand	(mg O_2/L)	70-2550	500-1200
Suspended solids	(mg/L)	50-940	250-850
Total nitrogen	(mg N/L)	7-36	20-85
Total phosphorus	(mg P/L)	2-27	6-20

Table 2. Ranges of raw waste water quality entering the treatment plant of Alcázar de San Juan (Central Spain, data gathered by the Guadiana Water Authority) compared to a typical domestic sewage (data reported by Pescod, 1992). The effluent of this facility often flows into Las Tablas de Daimiel National Park, 60 km downstream.

4. Abiotic effects

Waste water may enter freshwater ecosystems either in raw form or treated. In any case, water quality variability in sewage is noteworthy, as Table 2 and Figure 3 clearly depict. For example, total phosphorus concentration in treated waste water can experience high variations in periods of a few years (Fig. 3), ranging from 55 to 173% as CV in the effluent.

These strong variations in waste water inputs give rise to strong variations in pollution contents in the reception streams, which is also altered by streamflow fluctuations. Figure 4 shows the dramatic changes in total nitrogen and phosphorus concentrations in a semiarid river that has experienced waste water inputs since the early seventies in central Spain. If current European regulations of nutrient levels at the entrance of environmentally-protected areas were applied (*e.g.* total phosphorus contents in the waste water effluent lower than 2 mg P/L), this river would be demonstrated to experience at least one episode of strong pollution per year in recent decades.

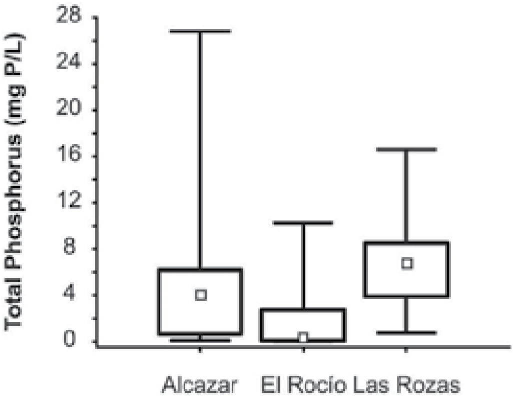

Fig. 3. Box-whisker plots of total phosphorus in effluents of waste water treatment facilities in semiarid Spain. Alcázar treated waste waters reach Las Tablas de Daimiel National Park, El Rocío treated waste waters enter Doñana National Park, and Las Rozas treated waste water are discharged to small brooks and streams running through the municipal territory of this city.

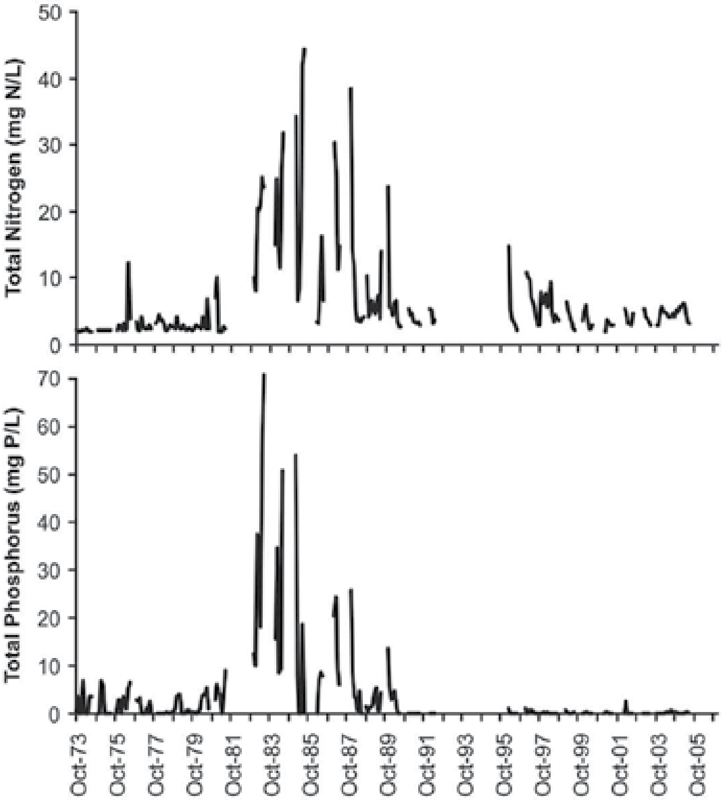

Fig. 4. Monthly total nitrogen and phosphorus concentrations in Gigüela river draining to Las Tablas de Daimiel National Park. The river is temporary and hence there are many periods in which it is dry, thus transporting no pollutants.

It is also well known that stream waters usually reduce their organic matter content through degassing (Cole & Caraco, 2001), denitrification (Piña & Alvarez-Cobelas, 2006) and burial in sediments (Masiello, 2004). Very few biogeochemical processes have been studied in semiarid freshwater environments as related with waste water; this is what older researchers termed as "self-purification". Our data indicate that, as a result of self purification, ranges of total phosphorus used as an index of waste water inputs decrease at the entrance of valuable freshwater ecosystems (compare Figs 3 and 5, Alcázar treatment plant-Las Tablas de Daimiel National Park, El Rocío treatment plant-Doñana National Park).

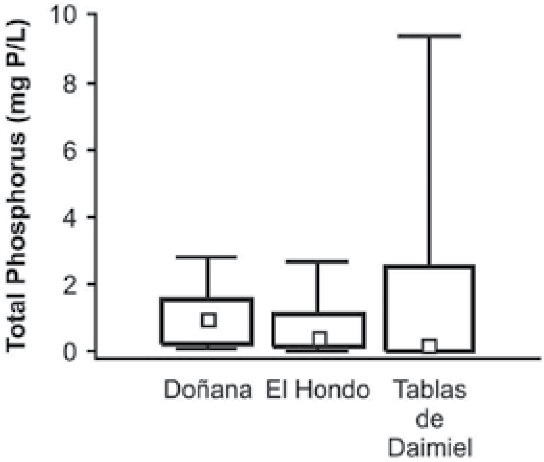

Fig. 5. Box-whisker plots of total phosphorus concentrations entering some Spanish freshwater ecosystems. Doñana wetland is in southwestern Spain, whereas El Hondo wetland is located in the SE. Las Tablas de Diamiel (central Spain) data is a much longer data set dating back to the eighties and hence its higher variability because waste water treatment was started in the nineties.

There are few data on biogeochemical effects of waste water discharge in semiarid freshwater environments. Some evidence reveals that it increases net heterotrophy of stream metabolism (Merseburger, 2006). Nitrogen-rich waste water appears to enhance both nitrification and denitrification downstream the point source of a treatment plant in semiarid streams (Merseburger, 2006; Fig. 6). Hotspots of chemoautotrophic activity may then appear as a result of waste water inputs (Merseburger et al., 2005). However, retention efficiency of nutrients has been demonstrated to be altered by the quantity and quality of waste water in Mediterranean semi-arid streams (Martí et al., 2004).

Once in the freshwater ecosystem, pollutant concentrations are strongly changed due to many biogeochemical processes (mineralization, burial, outgassing, uptake by organisms, even water abstraction for irrigation). This lead to high temporal variability of nutrient concentrations in rivers, lakes and wetlands, which is very important to understand its effects (Fig. 7). Because of the fluctuating nature of flow, the highest variability is perhaps experienced by stream systems (see the Arroyo de la Torre plot in Fig. 7), which cannot usually cope with waste water pollutants in episodes of low streamflow.

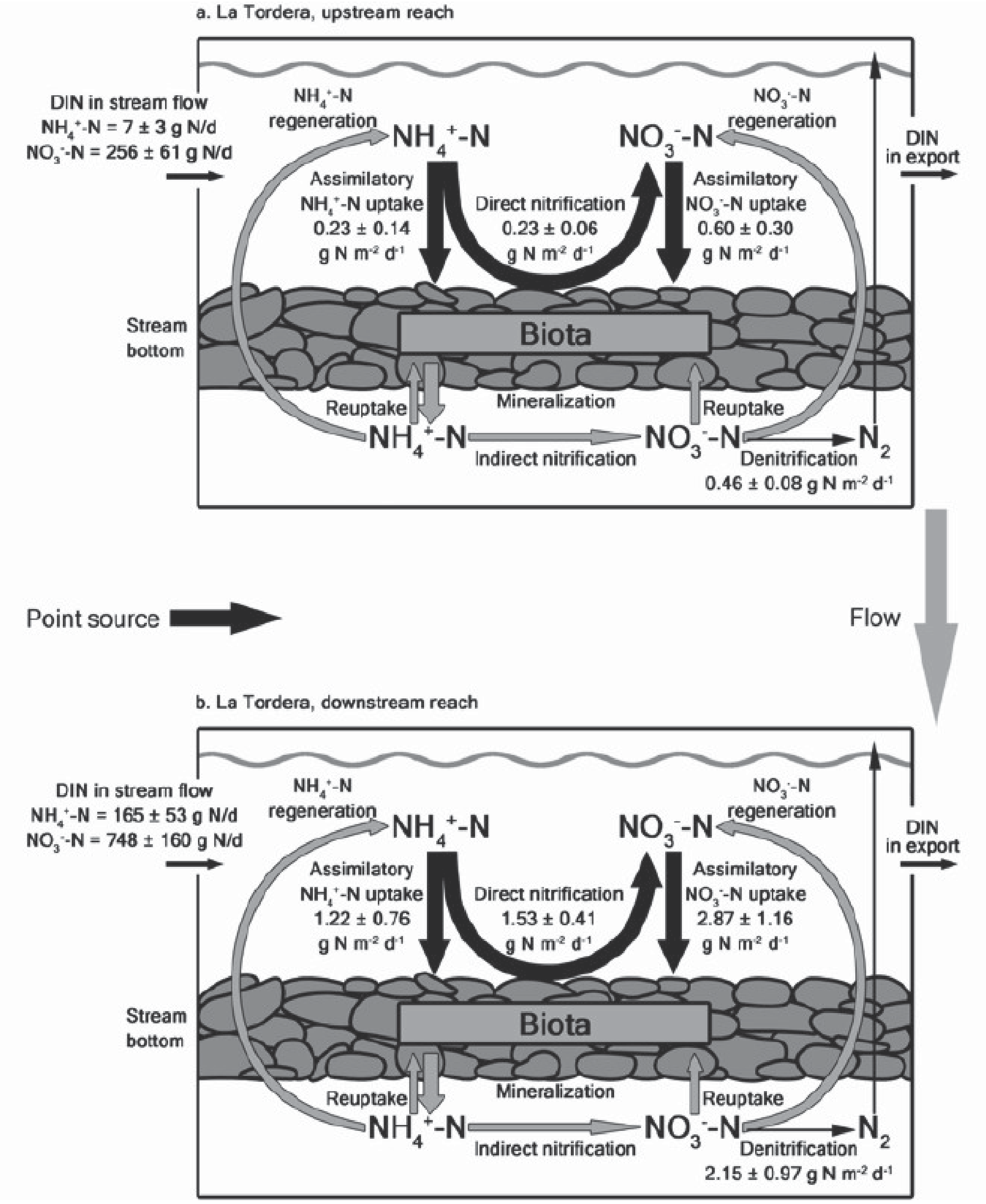

Fig. 6. Nitrogen processes in a forested catchment of NE Spain. The lower panel shows nitrogen dynamics downstream of the outlet of a waste water treatment plant (figure from Merseburger, 2006).

Phosphorus inputs of waste water promote primary production in freshwater ecosystems. The effects of phosphorus load has been the core of much research carried out in wetlands (Reddy & DeLaune, 2008), despite the fact that it rarely controls primary production (Sánchez-Carrillo et al. 2010). Our data for the hypertrophic wetland of Las Tablas de Daimiel National Park suggest that phosphorus is strongly retained in wetland

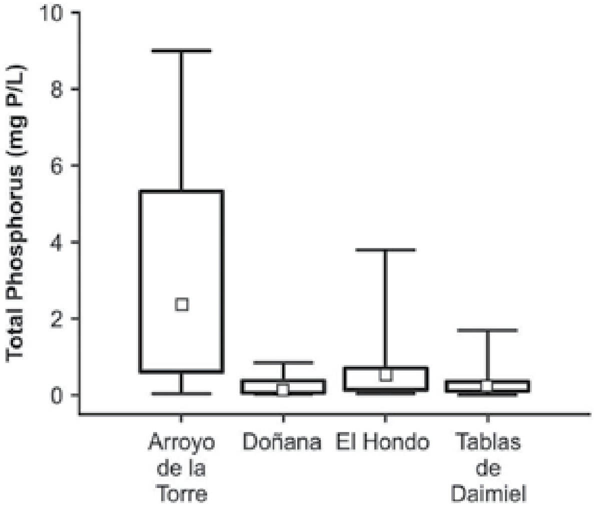

Fig. 7. Box-whisker plots of total phosphorus contents in some semiarid freshwater ecosystems of Spain. Arroyo de la Torre is an urban stream, whereas Doñana, El Hondo and Las Tablas de Daimiel are wetlands of varying salinity.

sediments (in central and outlet sites of the wetland, Fig. 8), which is later released to the water column during flooding cycles, increasing eutrophication. Although most of this element arises from wetland plant decomposition, phosphorus retention can reach up 85% of external inflows during humid periods (Sánchez-Carrillo & Álvarez-Cobelas, 2001). Spatial retention of phosphorus in semiarid wetlands is strongly linked to the kinetic energy of discharge, being lower at the inlet sites while increasing towards the outlet areas, following an exponential function (Sánchez-Carrillo et al., 2001).

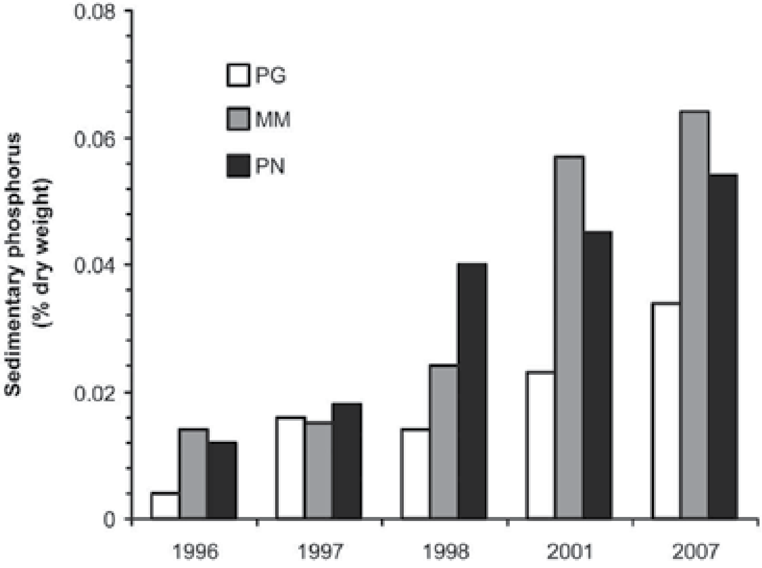

Fig. 8. Phosphorus content in sediments of Las Tablas de Daimiel National Park from 1996 onwards. PG is an inlet site, MM is a central site, whereas PN is an outlet site.

Very few data exist on stoichiometry of freshwaters as affected by waste water. Ours in the wetland of Las Tablas de Daimiel National Park reflect increasing N:P ratios towards the outlet (Fig. 9). What appears to be more interesting is that most areas show N:P ratios below 16 in less than half sampling dates, but the terminal site only shows these ratios in 15% of dates. This means that either nitrogen or phosphorus limitation of primary production might occur in many places of this wetland, except in the terminal area where phosphorus limitation may occur much more often, in spite of the pattern shown by phosphorus retention. Such a spatial distribution might reflect inputs of waste water whose N:P ratios are very fluctuating. Waste water inputs could often shift nutrient limitation of primary production from phosphorus to nitrogen in many areas of this wetland. Therefore, waste water may play an important, albeit poorly known, role in the stoichiometry of ecosystems that receive it.

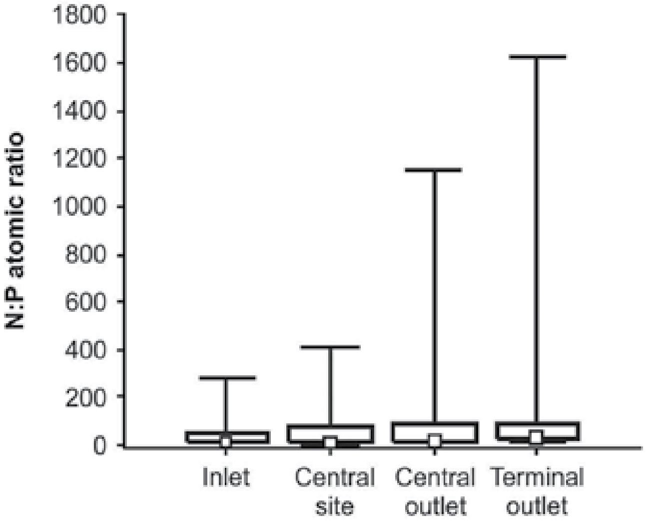

Fig. 9. Box-whisker plot of total nitrogen:phosphorus atomic ratio in some sites of Las Tablas de Daimiel wetland. Statistics have been calculated on monthly 1996-1998 data, a period when the wetland was continuously flooded, often receiving waste water. Distributions are not statistically different from each other, as judged by a Friedman test ($p > 0.05$).

Waste water also impairs the underwater light climate of freshwater ecosystems, strongly diminishing transparency, which in turn makes submerged plants disappear (Phillips et al., 1978). Long-term observations (> 30 years) in Las Tablas de Daimiel National Park by one of us (Cirujano-Bracamonte, unpublished observations) attest this statement; in the seventies and early eighties light penetration reached all depths in this otherwise shallow (< 4 m) wetland, but since early nineties most sites are turbid, with many suspended solids and dissolved organic carbon that turn water more turbid and brown-coloured.

The flowpath of waste water motion in semiarid freshwaters is another aspect that deserves mention, but has not been tackled as yet. This is particularly interesting in wetlands, where the patchy distribution of emergent vegetation enhances some directions of water motion, along with pollutants, and prevents others. One of us (S. Cirujano-Bracamonte) has often observed preferential flowpaths of waste water pollution in Las Tablas de Daimiel wetland, but this event has not been explored further. Clearly this pattern can increase the spatial heterogeneity of ecological effects of waste water.

All in all, it is clear that waste water dynamics in freshwater environments also relies on the quantity of water available. The interplay between water amount and the nature and concentration of waste water compounds is still poorly known. For example, the length of water renewal time is certainly related to biogeochemical dynamics of waste water, including its effects on changes of biological populations, but to our knowledge no study has been undertaken to support this. Retention time is related to water availability and the discharge:ecosystem volume ratio, but these key factors are seldom measured in semiarid environments, because 1st) it is not a simple task to measure discharge accurately in semiarid streams, 2nd) it is not easy to estimate ecosystem volume in semiarid freshwater environments which are very fluctuating, and 3rd) funding is often lacking.

Until now, we have dealt with chronic inputs of waste water, which are mostly of urban origin. Another entirely different topic is that of infrequent, but sudden inputs of waste water, which are the case for accidental pollution events. Obviously, they can hardly be predictable and hence it is impossible to implement sampling procedures and designs for them to study their magnitude and ecological effects. It is likely that these impacts are very different depending upon magnitude and stoichiometry, but we still have no data to support this.

5. Biological effects

Inputs of waste water to freshwater ecosystems usually result in changing species composition and this is the more studied feature of this topic (Liebmann, 1960-1962; Fjerdingstad, 1965; Sladecek, 1973). Diatoms and chrysophytes are usually substituted by green algae and cyanobacteria, most submerged macrophytes decline, many invertebrate groups (molluscs, copepods, cladocerans, water bugs, mayflies, caddisflies, most fish), disappear and others (oligochaetans, ciliates, rotifers, dipterans, carp, mosquitofish) colonize these impaired environments. Disappearances of many species have been reported in the long term study of Las Tablas de Daimiel National Park (Sánchez-Carrillo & Angeler, 2010).

Anyway, there are not many studies dealing with straightforward relationships between waste water and species richness of biological communities in freshwaters. It is often assumed that the effects of waste water are of complex nature, interacting with other environmental factors, and hence those relationships in semiarid ecosystems are seldomly reported. Our own work enables us to depict some of them. For example, it is a commonplace that the algal group of Euglenophytes is enhanced by organic matter (Sladecek, 1973); our data for the inlet area of Las Tablas de Daimiel National Park, which receives waste water very often (see Fig. 4), suggest that this holds true, because chemical oxygen demand almost explains half of the whole variability in species richness of this algal group (Fig. 10).

Waste water can also have strong effects on aquatic productivity. Phytoplankton biomass at the inlet of Las Tablas de Daimiel National Park often exceeds 200 mg Chl-*a*/m^3, with production values that can be close to the highest ever recorded (Alvarez-Cobelas et al., in press; Talling et al., 1973). Depending upon their ecological performance, the cover of emergent plants may reflect the impact of waste water. While reed (*Phragmites australis*) cover over the years is enhanced by phosphorus, cut-sedge (*Cladium mariscus*) cover is impaired (Fig. 13).

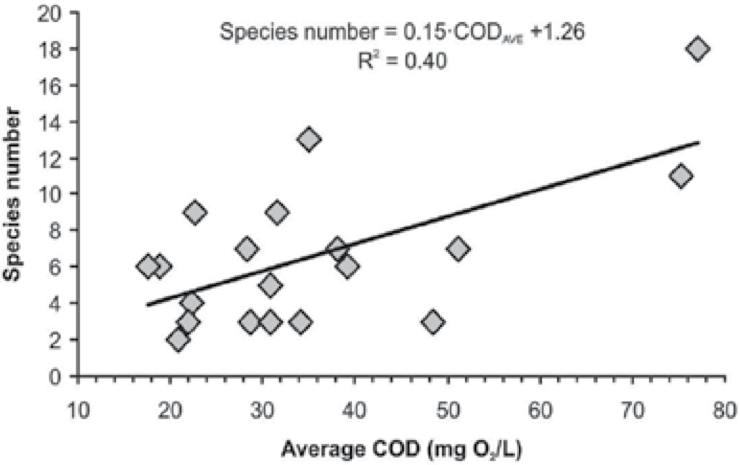

Fig. 10. Species numbers of Euglenophytes as related with average Chemical Oxygen Demand (COD) at the main inlet of Las Tablas de Daimiel National Park. Data from 1996-2002, reported in Conforti et al. (2003). The relationship is statistically significant at $p < 0.05$.

If the whole community of riverine benthic algae and macroinvertebrates is considered, the higher the chemical oxygen demand the lower the species richness of these biological groups (Figs. 11-12).

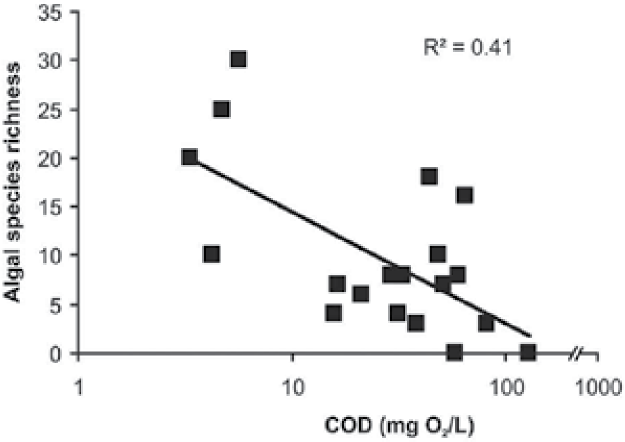

Fig. 11. Species richness of benthic algae in streams of Las Rozas de Madrid (central Spain) watershed as related with chemical oxygen demand (COD) in a semilogarithmic plot. Data from 2004-2006.

Plants living on the surface of lakes and wetlands, such as the aquatic fern *Azolla* and the water lentil *Lemna*, are enhanced by total phosphorus concentrations in water. Our study of this invasive fern in Doñana National Park has demonstrated a strong relationship between phosphorus and the biomass that it can attain, though other less known factors may be important for *Azolla* growth at times (Fig. 14).

Lemna (Fig. 15) has often occupied big surface areas in Las Tablas de Daimiel National Park over the years, often attaining large covers, as flooding water and high phosphorus concentrations have enhanced its development.

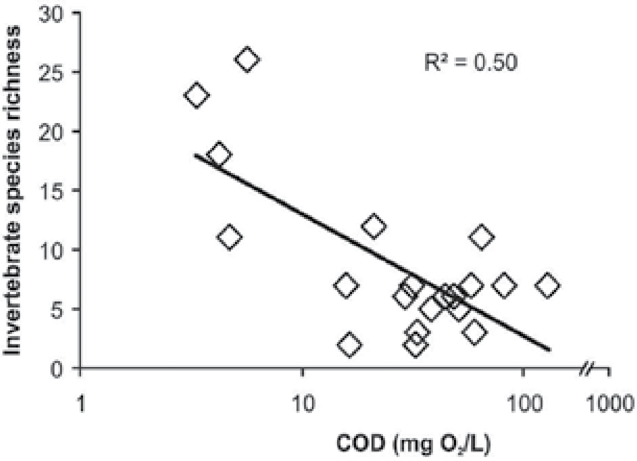

Fig. 12. Species richness of benthic macroinvertebrates in streams of Las Rozas de Madrid (central Spain) watershed as related with chemical oxygen demand (COD) in a semilogarithmic plot. Data from 2004-2006.

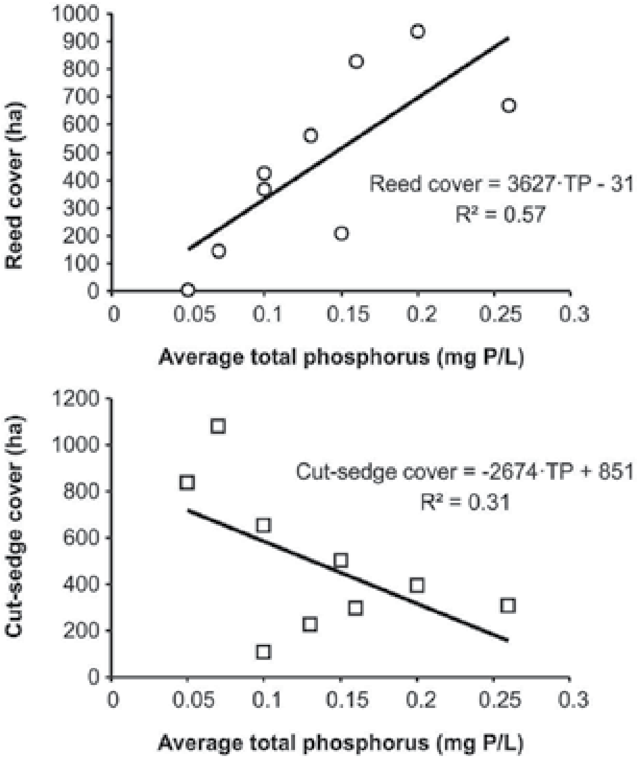

Fig. 13. Cover of dominant emergent helophytes in Las Tablas de Daimiel National Park over the years as related with average total phosphorus concentrations in the wetland. Data recorded in scattered years from 1956 until 2007. Both relationships are statistically significant at $p < 0.05$.

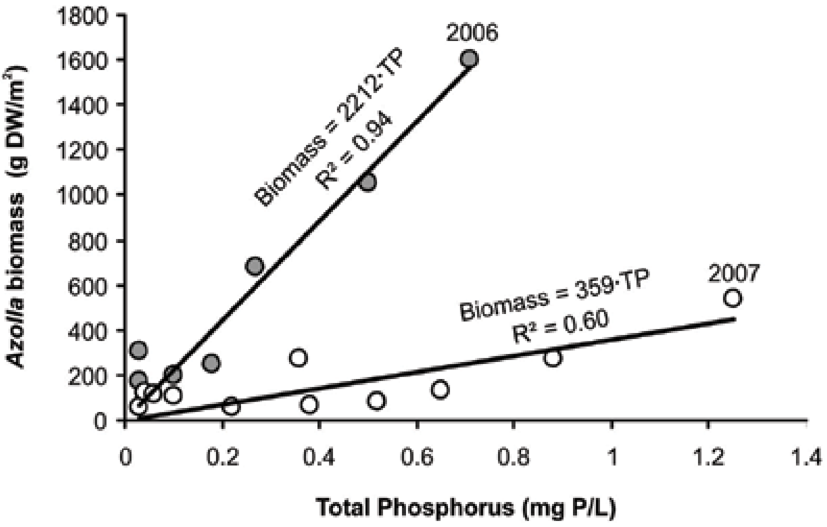

Fig. 14. Biplots of total phosphorus concentration and *Azolla* (an aquatic fern) dry biomass in the wetland of Doñana National Park in two years of contrasting flooding. All relationships are statistically significant ($p < 0.01$).

Fig. 15. Surface occupation of Las Tablas de Daimiel National Park by *Lemna gibba*, the water lentil, in 1996 (photograph by S. Cirujano-Bracamonte).

The synergistic impact of factor interactions is often more important than the impact of a single factor, such as phosphorus for example. In this context, semiarid environments are also impacted by unevenness of water availability, sometimes an outcome of anthropogenic nature. This can be worsened if water renewal is lowered by smaller discharge, and decomposing organic matter of primary producers remains in the ecosystem. Our studies have identified such an interplay for many species in Las Tablas de Daimiel National Park, which has caused many species disappear because of strong water shortage and impaired water quality of autochthonous origin (Table 3; Alvarez-Cobelas et al., 2001). Freshwater mussels and the fairy shrimp have also been extinguished in that marshland by such an interplay of factors.

	1956	1973	2007
Emergent macrophytes	18	18	11
Submerged macrophytes	22	20	5
Fish		16	3
Ducks and herons		21	16

Table 3. Species richness of the most conspicuous biological groups in Las Tablas de Daimiel National Park over time. From the seventies, the combined action of shorter water availability, arisen from unsustainable irrigation, and impaired water quality has resulted in diminishing species richness. Data source: Alvarez-Cobelas et al. (2001) and Sánchez-Carrillo & Angeler (2010).

6. Remedial actions

Semiarid environments are mostly located in areas of moderate economic development. In addition, the ongoing, and unfortunately occurring on a long-term basis, world economic crisis will threaten funding for environmental management and ecosystem restoration. This crisis is worsened in many Mediterranean countries, such as Spain and Greece, as a result of the decay of the building industry and the adaptation measures of their governments that intend to save public investments that enable payments of the extraordinarily high debt rates owed to international banks. Otherwise, there would be a risk of national bankruptcy for these countries. Therefore, it is likely that less and less money will be devoted to environmental protection of these nations in the years to come, which will make environmental restoration of waste water-impacted ecosystems very doubtful.

On the other hand and with a low degree of uncertainty, water will be more scarce as a result of global warming in semiarid climates (Parry et al., 2007). It is thus likely that waste water will often be the single water source for many freshwater environments as time goes by. All these conditions, *i.e.* less water available and of lower quality and lower funding for environmental management, must make us think of simple, green and money-saving environmental technologies to confront these challenges. Raw waste water cannot enter as such in rivers, wetlands and lakes of semiarid countries, because if so they will be either impaired very soon or hardly restored. Treated waste water is unfortunately the only solution to secure water availability enough for ecosystem performance. It is then urgent to develop treatment systems adapted to the peculiarities of the semiarid regions. Probably, treatment wetlands upstream of valuable freshwater ecosystems to diminish the impact of nutrient-rich streamwater, is the best option and the more economically feasible in the short term. The technology for such wetlands is now widely available; they are not expensive and can be operated by native, non-experienced workers (Kadlec & Knight, 1996), and probably the only one that can cope with the strong fluctuating hydrology of semiarid climate.

Other measures to cope with these problems, such as water saving, water reuse and lower consumption of commodities that decrease pollutants exported to river courses, seem unrealistic, given the culture of luxury consumption of goods and resources installed in many developed semiarid societies (Davis, 1977).

7. Future research

There are still many aspects that deserve attention because they have hardly been tackled as yet. As a general recommendation, we suggest specifically designed studies on the relationship between waste- and freshwaters. Up to date, waste water is studied by waste water treatment engineers and freshwater is studied by ecologists, without much exchange of knowledge among both groups of people. Joint studies have to be carefully designed if our knowledge on this topic is to be expanded, increased and improved. This is particularly important in water shortage scenarios such as those of semiarid countries where a culture of collaboration among scientists and technicians is almost lacking.

Another suggestion is to pay more attention to the synergistic features of interplaying factors. Waste water effects are complicated by climate patterns and hence by water availability and renewal effects, but this is rarely considered in semiarid countries, where quantity and quality management offices do not perform collaborative work.

More specifically, effects of waste water on freshwaters have always been searched from the statistical viewpoint, mostly relying on the correlation approach. It is now time to focus on another approach, the cause-effect approach planning carefully designed experiments to fully address and describe those effects.

The biogeochemistry of interacting waste- and freshwater has scarcely been explored. Not only stoichiometry issues have to be studied, but also many processes that have been studied in climatic areas other than the semiarid one deserve attention. Among these, there are the effects of uneven patterns of wet-dry cycles in nutrient enriched sediments exposed to waste water discharges, the resilience of nutrients and other toxic substances in the aquatic environments, the methane, carbon dioxide and nitrogen gas emissions (which can be enhanced by waste water inputs), the sulphate reduction process and many more. Even the anammox process, which has originally been described from waste water treatment plants (Mulder et al., 1995), is likely occurring in freshwaters impacted by nitrogen-rich waste water (Zhu et al., 2010), but has not been searched for as yet.

Trophic cascades have not been considered as related to waste water inputs, and they should be because waste water usually kills most fish faunas, but when diluted, enhance carp occurrence. Carps also increase internal loading by bioturbation of sediments (King et al., 1997), thus affecting the survival of many benthic invertebrates and plants.

Concerning novel tools, there are some whose use must be rewarding. Microbial communities are very important mediating chemical transformations of waste water, and restriction enzymes of nucleic acids can be profitably used for a thorough description of bacterial communities, which otherwise is almost lacking in semiarid freshwaters. Stable isotopes, which have been used to trace the origin of waste water (Cole et al., 2004), could also improve our knowledge of carbon, nitrogen and phosphorus cycling is freshwaters impacted by raw or treated waste water. Specifically, the study of O^{18} signature within the PO_4 ion will aid in outlining the flow path and transformations in freshwater of waste water-derived phosphorus, following the studies pioneered by McLaughlin et al. (2006).

8. Conclusion

Raw or treated waste water is very often discharged to freshwaters and results in changing ecological performance and biological diversity of these systems. The problem is particularly acute in semiarid regions where the beneficial effect of dilution is impaired by longer water retention time that arises from reduced rainfall and more water requirement for irrigation as compared with those of freshwaters in other latitudes. Some data suggest that waste water might change stoichiometry of freshwater ecosystems. Waste water inputs have been shown to be straightforwardly related with species richness of some algae (Euglenophytes), protozoa (Ciliates) and insects (Diptera), and inversely with species richness of most biological assemblages. Phytoplankton biomass and productivity and the biomass of some higher plants, such as *Lemna* and *Azolla*, are also enhanced by waste water. The cover of some species of emergent vegetation in semiarid wetlands, such as *Phragmites*, benefits from waste water inputs. Despite these findings, our knowledge on effects of waste water on freshwater ecosystems in semiarid regions is still very limited and hence must increase to prevent noxious and/or unwanted changes in these ecosystems.

9. Acknowledgements

Most data and studies reported here could not be available without the generous support by the Spanish Network of National Parks to our research group over the decades. More specifically, Projects 81/2005 and 01/2008 of this Agency have funded our more recent studies. The Guadiana Water Authority has also provided us with support and long-term data.

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11

Hydrochemical Investigation of Groundwater Contamination in the Grombalia Shallow Aquifer, Cap Bon Peninsula, Tunisia: Impact of Irrigation with Industrial Waste Water

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1. Introduction

During the last two decades, demands for groundwater from urban, industrial development and extensive agricultural activities in the Grombalia basin, Cap Bon peninsula, north-eastern Tunisia, particularly in the Beni Khalled, Menzel Bou Zelfa and Soliman regions have resulted in increased withdrawals from the Quaternary shallow aquifer. As an example of water supply related problem generalised water level decline and the deterioration of groundwater quality. In deed, in recent times, soils have become increasingly polluted by waste water and agricultural chemicals (fertilisers, pesticides, herbicides). In shallow groundwaters this pollution can easily be transported.

The major economic role of the shallow aquifer has raised concerns relating to the effects on groundwater resource as (i) the recharge rate of the shallow aquifer is not known with precision and (ii) the detrimental effect on the environment in relation with the groundwater contamination and salinization, which put a strain on the existing fresh water that supports the regional development. Subsequently there is a requirement for agreed and consistent examination and assessment activities to recognize the source of the pollution and evaluate its current amount and future expansion. It's within this framework that is undertaken the present study, which aims to provide reliable information about the hydrochemical characteristics of groundwater and the main groundwater mineralization processes. It also investigates the impact of regional agricultural and industrial activities on groundwater quality.

2. Study area

The study area belongs to the NW–SE troughs domain of the Cap Bon Peninsula, north-eastern Tunisia and cover an area extent of about 720 km² (Elmejdoub and Jedoui 2009). It is boarded to the north by the Gulf of Tunis and the Tekelsa Hills, to the east by the Abderrahman Mountain and the oriental coastal highlands, to the south by the Hammamet Hills and to the west by the Bou Choucha and the Halloufa mountains (Fig. 1). The climate of the study area is classified as Mediterranean, semi-arid; with mild, wet winters and warm, dry summers. The average annual rainfall ranges between 500 and 600 mm (Ben

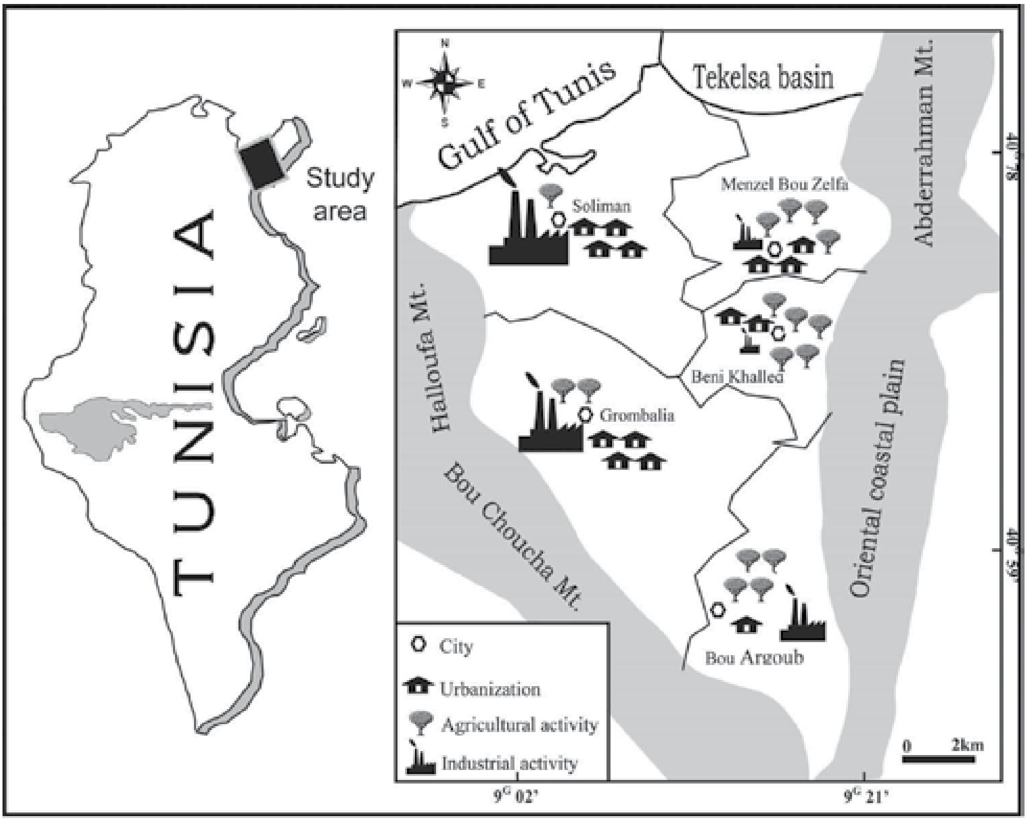

Fig. 1. Location map of the study area

Moussa 2007). About 80% of this precipitation occurs between September and March. The potential evapotranspiration is about 1,200 mm year-1. The average annual temperature is about 18°C with maximum temperatures up to 30°C in summer. Regionally, the surface drainage is toward the north reflecting regional topographic gradients. It is constituted by several ephemeral Wadis, which collect surface runoff from the surrounding highlands toward the Gulf of Tunis.

3. Geology and hydrology

The Grombalia Basin is located in the south-western part of the Cap Bon Peninsula, which is situated astride the African–Eurasian plate boundary (Elmejdoub and Jedoui 2009). Geologically, it is described as a graben oriented NW–SE and filled by Quaternary sediments. The edges of this graben were related to two normal faults that appeared during the Middle Miocene (Hadj Sassi et al. 2006). These are the Borj Cedria NNW–SSE normal fault and the Hammamet NE–SW normal fault (Ben Ayed 1993; Ben Salem 1995; Chihi 1995). The sedimentary units outcropping in the basin are represented by recent Quaternary soil and terraces that partially cover the Eocene, Oligocene and Miocene Formations. The Eocene deposits are mainly constituted of Glauconeous sands of the Souar Formation, which locally outcrops in the north of the Halloufa Mountain. The Oligocene unit is principally made up of coarse to medium-grained sandstone belonging to the Fortuna Formation

(Burollet 1956; Blondel 1991). It largely outcrops along the Halloufa and Bou Choucha mountains in the western part of the basin. The Miocene sandstone and clay series are found essentially in the Oriental costal hills and in some restricted areas along the foot of the Halloufa and Bouchouch mountains. The Quaternary detrital sedimentations of the Rejich Formation mainly consist of fine to coarse-grained sands, clayey sands, sandstone, silt and abundant evaporate deposits (Schoeller 1939; Colleuil 1976; Ben Salem 1995).
From a hydrogeologic point of view, the Grombalia alluvial aquifer that is characterized by an average thickness of about 50 m is hosted in the Quaternary continental sand, clayey sand and sandstones deposits, which repose on a 15 m-thick clayey bed-rock (Fig. 2). The potentiometric map, realized on the basis of water level measurements of 41 wells, displays the main groundwater flow patterns in the Grombalia unconfined aquifer. It shows that the recharge occurs in the pediments of the surrounding mountains and converges to the central part of the basin. There, a general southeast–northwest flow carries groundwaters to the Gulf of Tunis discharge areas (Ben Moussa 2007). The exploitation of groundwater from the Grombalia shallow aquifer started in the 1950s; the number of shallow wells increased through time. At present, this number exceeds 11,000, with a total extraction of 249 Mm3 (DGRE 2005). Consequently, the water level has dropped between 1 and 10 m, with an annual rate of about 0.3 m.

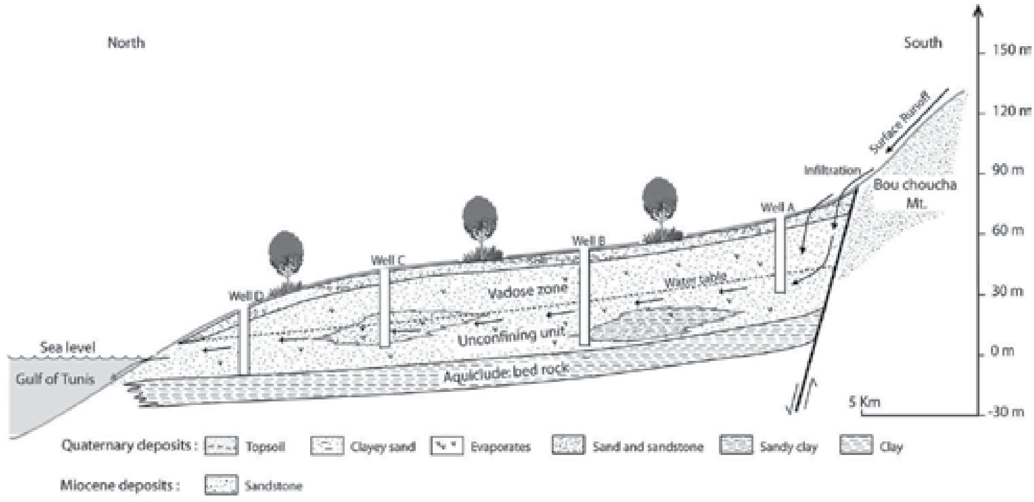

Fig. 2. Hydrogeological cross section of the Grombalia unconfined aquifer

4. Sampling and analytical procedure

A field sampling campaign was carried out during September 2005. A total of 38 groundwater samples was collected from dug wells tapping the shallow aquifer at depths ranging between 6 and 35 m. In situ measurements of pH, Temperature, electric conductivity (EC) and the total dissolved solids (TDS) were performed in the field. Water samples were filtered and collected in 100 ml polyethylene bottles with poly-seal caps for major and minor elements analysis which have been done at the ''Laboratoire de Radio-Analyses et Environment'' of the ''Ecole Nationale d'Ingenieurs de Sfax'' (Tunisia), using titration method (for HCO_3) and standard ion chromatographytechniques.

5. Results and discussion

5.1 Partial pressure of carbon dioxide (pCO_2) and in situ measurement

The pCO2 values, calculated with WATEQ4F program (Ball & Nordstrom, 1991), in situ parameters, total dissolved solids (TDS) and analytical data of the major and minor ions in groundwater samples are measured. The pCO_2 values range from 0.29 10^{-2} and 4.63 10^{-2} atm. The highest contents of pCO2 characterize wells located downstream suggesting an increasing along flow path. The lowest values are registered in the recharge area, which signify that fresh groundwater displays lower pCO_2 levels upstream the basin close to the recharge area and in the vicinity of the Wady courses. However, groundwaters with higher pCO_2 levels provide insight into the more extensive water rock interaction and the microbial mediated reactions that produce CO_2 (Rightmire, 1978; Adams et al., 2001). Groundwater samples show relatively heterogeneous values of temperature, varying between 13.9 and 21.2°C. Relatively high temperatures, close to that of the atmosphere, characterize Soliman and Menzel Bou Zelfa regions. These high temperatures are probably related to the shallow depth of the groundwater table. However, deeper wells of Grombalia, Beni Khaled and Bou Argoub regions are distinguished by relatively low temperatures, indicating that they are not or slightly influenced by the atmosphere. Groundwater samples, which are almost neutral, show very homogeneous pH values ranging between 6.9 and 7.8. The EC values vary in a wide range from 1.1 to 7.8 mS/cm that lend support to the interference of numerous natural and anthropogenic processes.

5.2 Origin of groundwater mineralization

5.2.1 Water type

The Piper diagram (Piper 1944) has been established in order to precisely specify the water types in the Grombalia shallow aquifer. Nitrate concentration was taking into account when plotting this diagram because of its relative abundance in the groundwater (Fig. 3). The data plotted in Piper classification diagram display show the same Na–Cl–NO_3 water type.

5.2.2 Nitrate

The nitrate contents in the studied aquifer show a large range of variation, from 0 to 384 mg/l. Ninety percent of the groundwater samples taken during this study, show nitrate concentrations exceeding the maximum European admissible nitrate concentration limit in drinking water (50 mg/l). The examination of the nitrate distribution map (Fig. 4) reveals that high nitrate concentrations appear to be related to agricultural land-use patterns and suggests that the application of nitrogen fertilisers and the irrigation with treated waste water lead to increased nitrate leaching. The areas with nitrate concentrations, exceeding 50 mg/l, are located in the Soliman and Beni Khaled region where agricultural activities are more developed (Fig. 5). This may reflect the influences of agricultural practices that introduce a long-term risk of groundwater pollution by over-fertilization and pesticides leached downward in conjunction with the excess use of treated waste water, particularly in the Soliman region. In fact, the nitrification is likely related to the return flow phenomenon as the most enriched samples were collected from the agricultural areas. In this region, where flood irrigation is applied at a large-scale, the excess of irrigation water undergo excessive nitrate enrichment at the ground surface; after that it eventually returns to the unconfined aquifer. Indeed, the positive relationship between NO_3 and SO_4 (Fig. 6) suggests that both N and S are used in the study area in the form of $(NH_4)2SO_4$-fertilisers (Bohike et

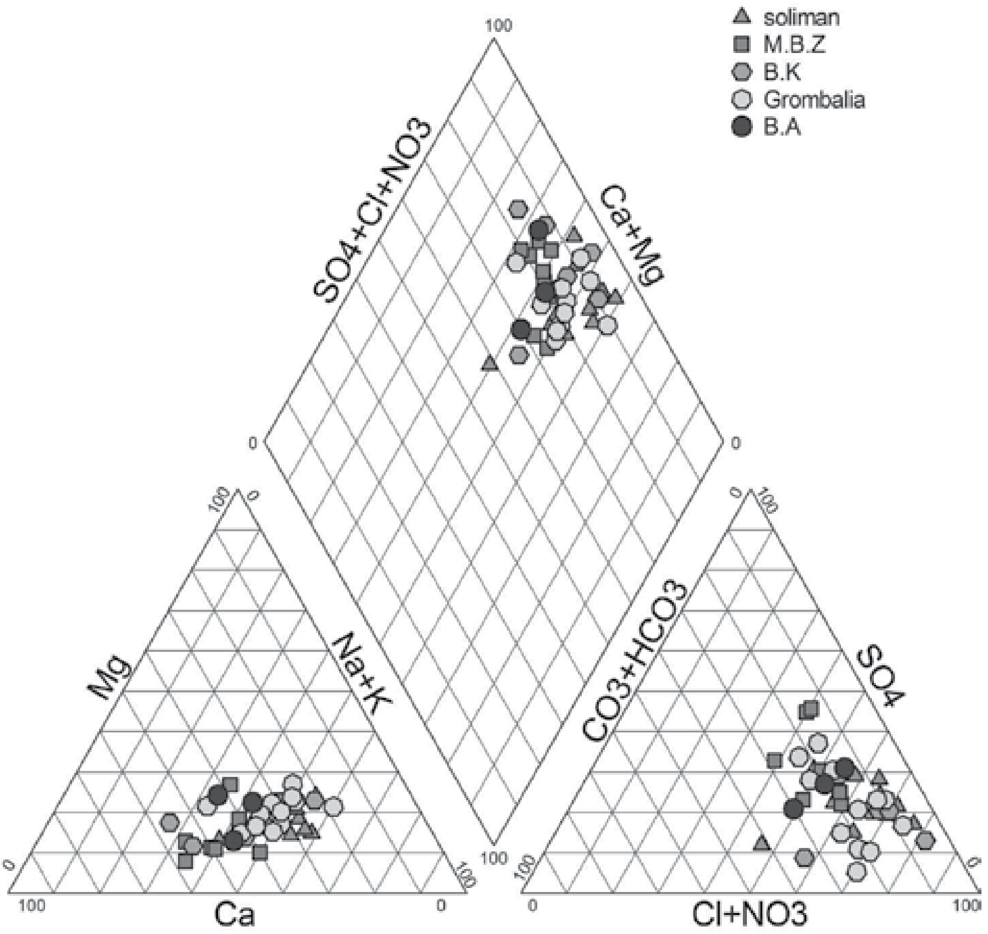

Fig. 3. Piper diagram of the Grombalia unconfined aquifer

al., 2007). Moreover, some groundwater samples show a well-defined relationship between NO_3 and Ca (Fig. 7), highlighting that both elements are mostly originated from the excessive use of $Ca(NO_3)_2$-fertilizers (Stigter et al., 2006). Therefore, the nitrate contamination is a result of the local hydrogeological setup coupled with the traditionally applied flood irrigation and the complete lack of environmental awareness regarding the over-fertilisation and the utilisation of recycled waste water.

5.2.3 Strontium and bromide

Strontium and bromide are generally used as tracer due to their geochemical characteristics, in particular when they are used in conjunction with other conservative elements such as chloride. These elements are relatively abundant in the Grombalia shallow groundwater and they are used to identify sources of groundwater contamination as well as hydrogeochemical reactions accompanying salinization processes.

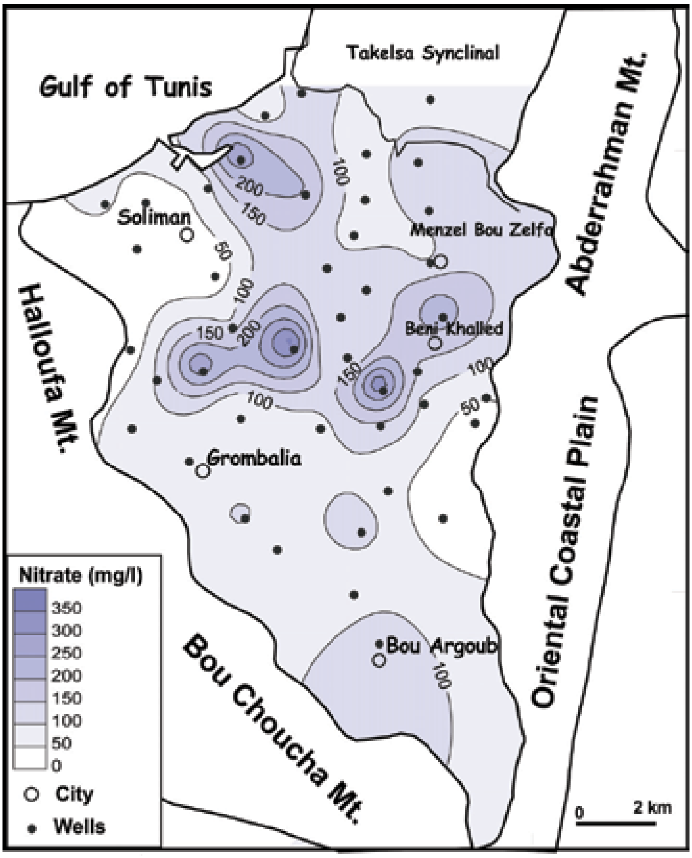

Fig. 4. Spatial distribution of nitrate

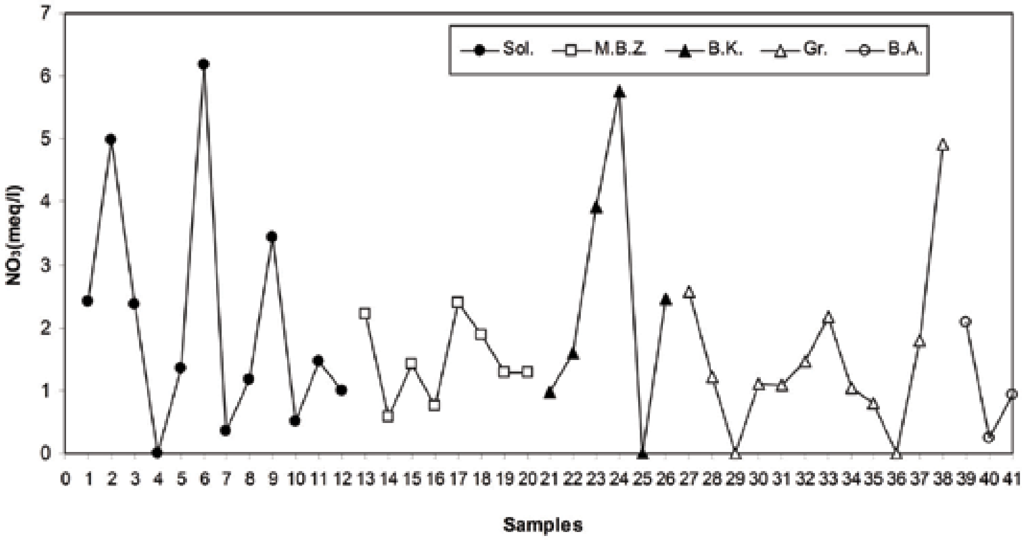

Fig. 5. Regional evolution of nitrate concentrations

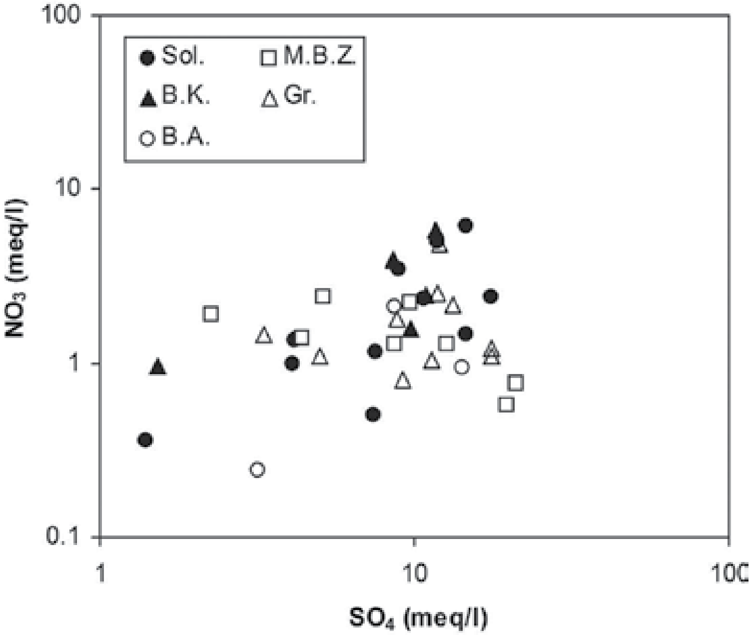

Fig. 6. Plots of SO_4 *vs*.NO_3

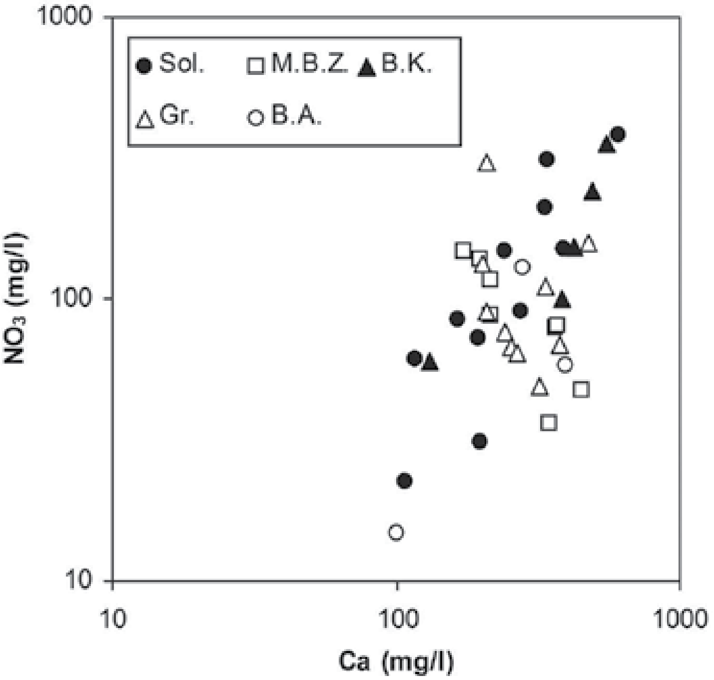

Fig. 7. Plot of Ca *vs* NO_3

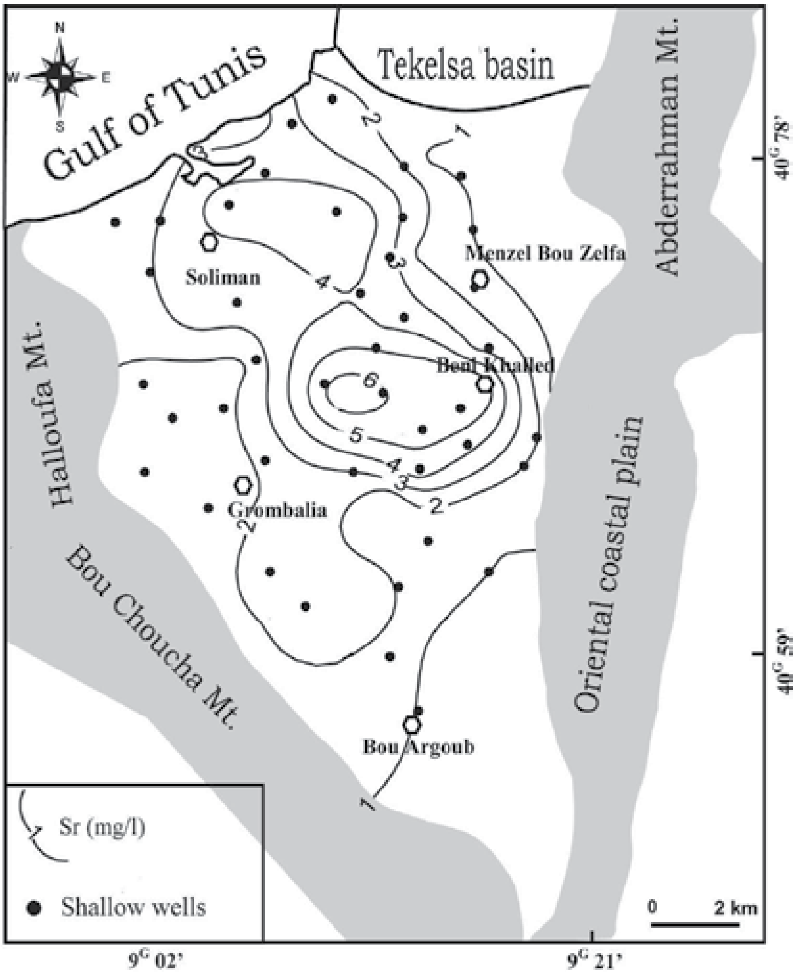

Fig. 8. Spatial distribution of stontium

The strontium concentrations in the Grombalia shallow aquifer vary within a large range from 0 to 1.5 mg/l. High strontium concentrations, exceeding 1.5 mg/l, characterize the central-western part of the basin (Fig. 8). However, moderate Sr contents are registered in Soliman and Beni Khaled regions; and relatively low contents distinguish Menzel Bou Zelfa and Bou Argoub regions (Fig. 9). High and moderate strontium concentrations, which characterize the Grombalia, Soliman and Beni Khaled regions, are mainly related to downward leakage in relation with return flow from irrigation with treated industrial waste water and, to a negligible extent, to the interaction with sulphate minerals. The correlation of SO_4 versus Sr (Fig. 10) shows a positive relationship, which indicates some Sr contribution from dissolution celestite ($SrSO_4$) associated with gypsum (Faye et al. 2005). While, the return flow is strongly suggested considering the relatively low depth of the shallow aquifer water table in the mentioned regions. On the other hand, in the Menzel Bou Zelfa and Bou Argoub zones, the ratio of strontium to sulphate is in disagreement with the celestite dissolution. In the Bou Argoub region, industrial non-treated waste waters are directly discharged into the drainage networks that may provoke high strontium contents.

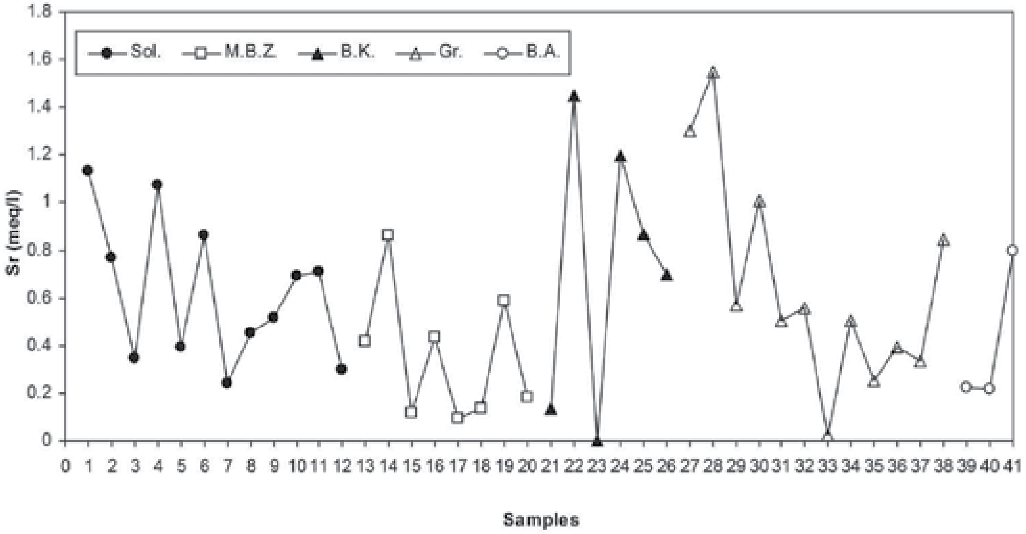

Fig. 9. Regional evolution of strontium concentrations

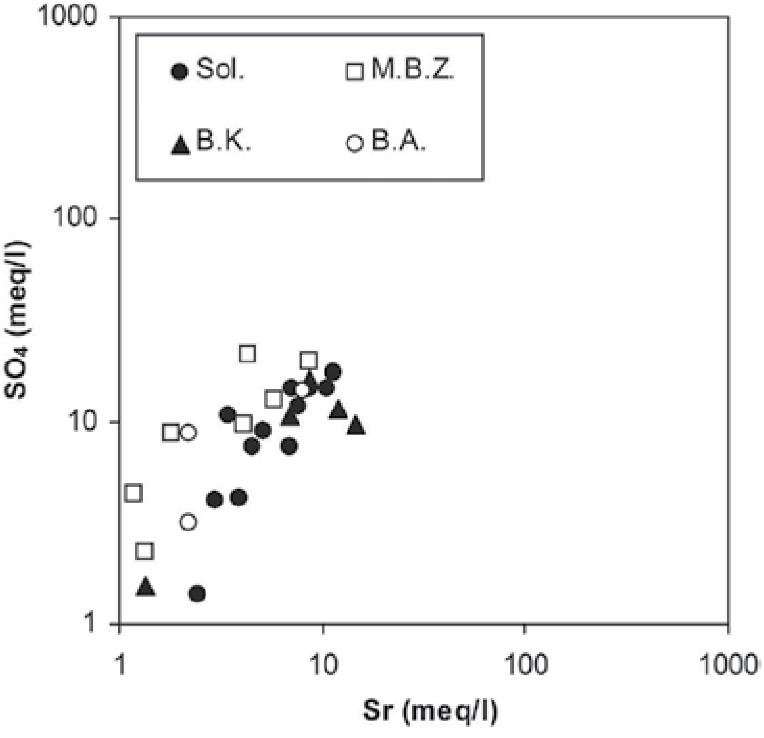

Fig. 10. Plot of Sr *vs* SO_4

Groundwater samples collected from the studied shallow aquifer show bromide contents ranging between 3.7 and 9.3 mg/l (Fig. 11). These bromides concentrations, which are above the admissible limit for drinkable waters, are too high to be derived from natural sources i.e. evaporate dissolution and marine origin of bromide. In deed, waters with high Br concentrations are localized in the central part of the basin (in Grombalia and Beni Khaled regions) but not in the Soliman costal region. This may indicate that high Br contents are so far to be related to natural sources. Consequently, these high Br concentrations are probably related the agricultural land-use patterns and lend support to the significance of the contamination by rejection of treated and non-treated waste water; and return flow from irrigation water that include high amounts of fertilizer and pesticides such as methyl bromides and other organic bromyl (Zhu et al. 2007).

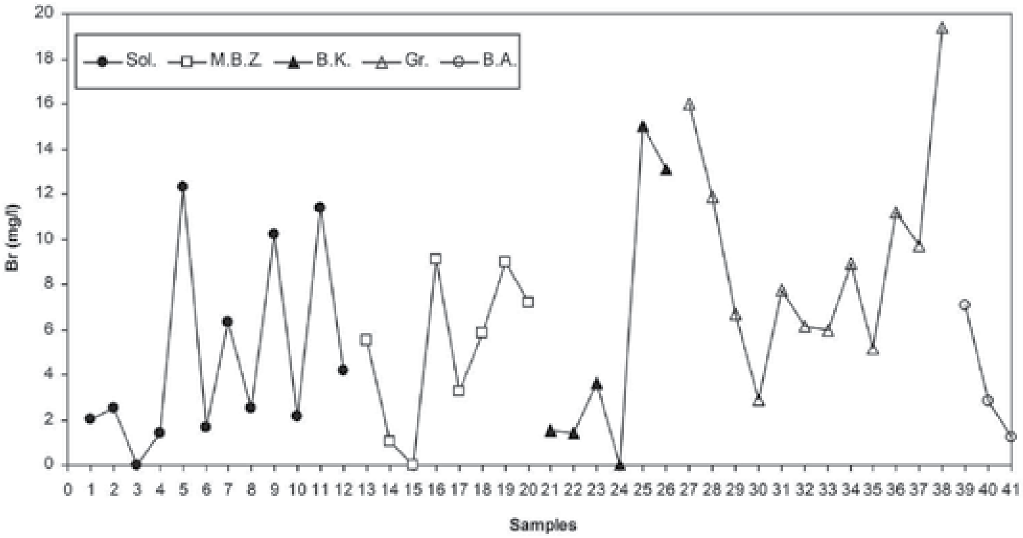

Fig. 11. Regional evolution of bromide concentrations

In addition to the anthropogenic mineralization processes mentioned above, natural processes play also a significant role in the contamination of groundwater in the Grombalia shallow aquifer.

Bivariate diagrams between ions are useful because they can point out associations between elements that can show the overall coherence of the data set. They can also indicate the participation of the individual chemical parameters in several influence factors, a fact which commonly occurred in hydrochemistry. In the plot of Na/Cl (Fig. 12), all groundwater samples fall on the 1:1 (Na:Cl) line. This well-defined correlation in conjunction with the undersaturation state with respect to the NaCl argue for the role of halite dissolution as a major process contributing to the groundwater salinization (Appelo and Postma, 1993). The dissolution of halite is verified through the general increasing trend in the Na and Cl concentrations along the groundwater flow direction (Fig. 13). The positive correlation between Ca and SO_4 (Fig. 14) and their similar spatial distribution (Fig. 15), which increase towards the discharge area, reflect that the dissolution of gypsum is another significant salinisation process. Indeed, saturation indexes show that some samples are under-saturated with respect to gypsum and anhydrite, highlighting a geochemical condition dominated by

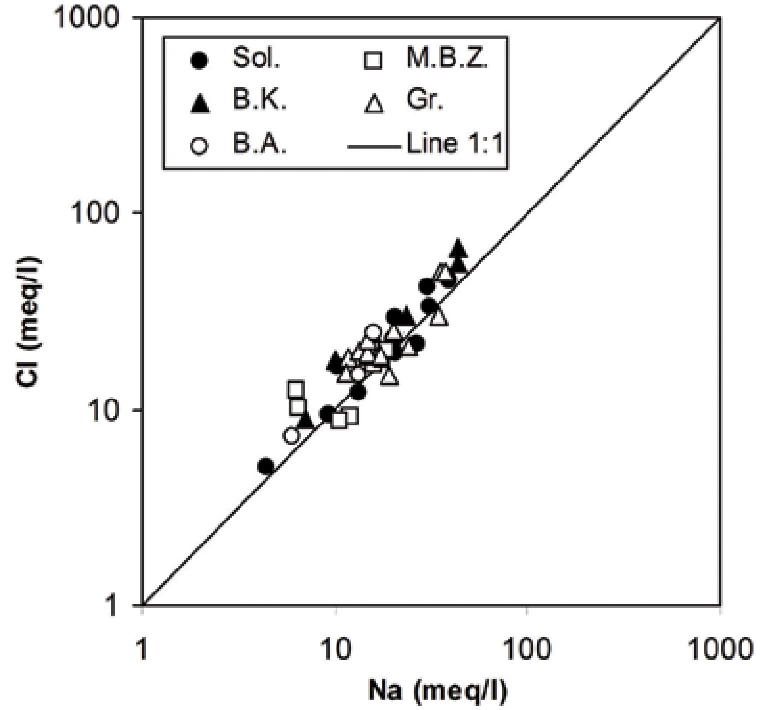

Fig. 12. Plots of Na *vs* Cl

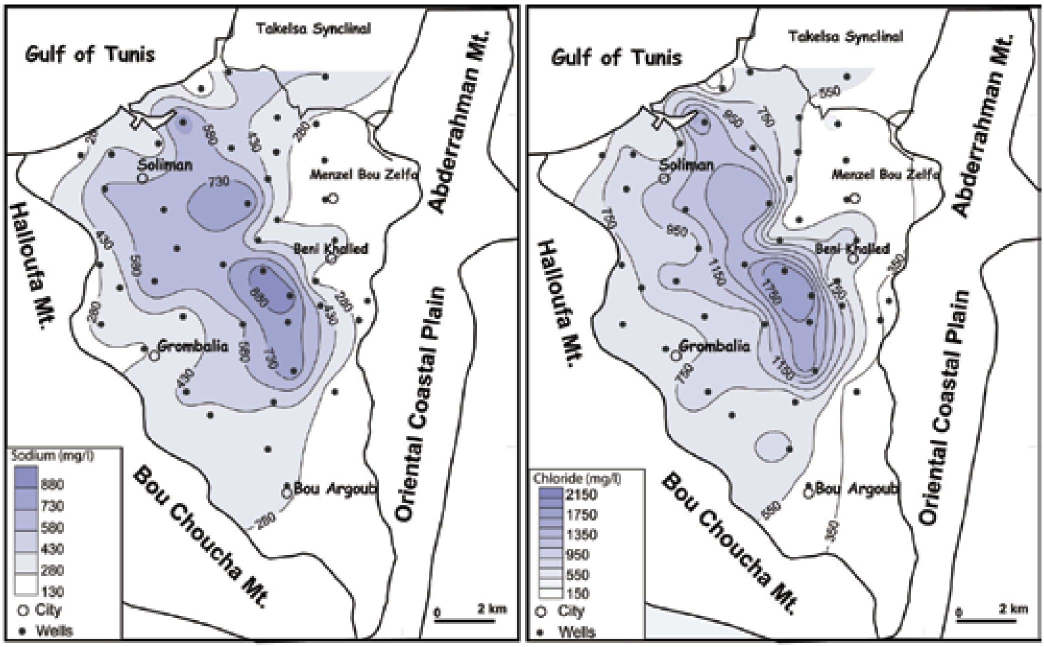

Fig. 13. Spatial distribution of Na and Cl

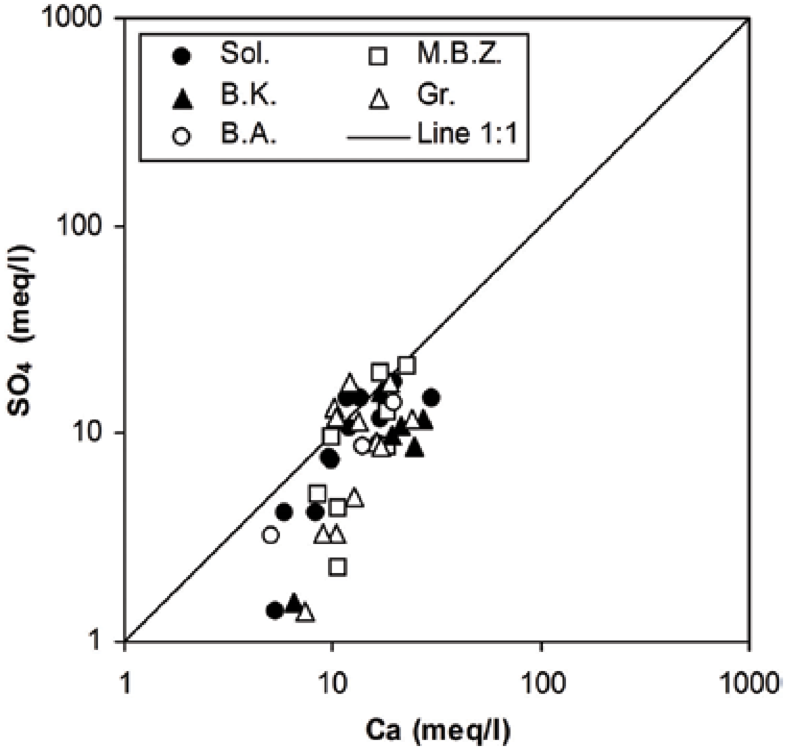

Fig. 14. Plots of Ca *vs* SO_4

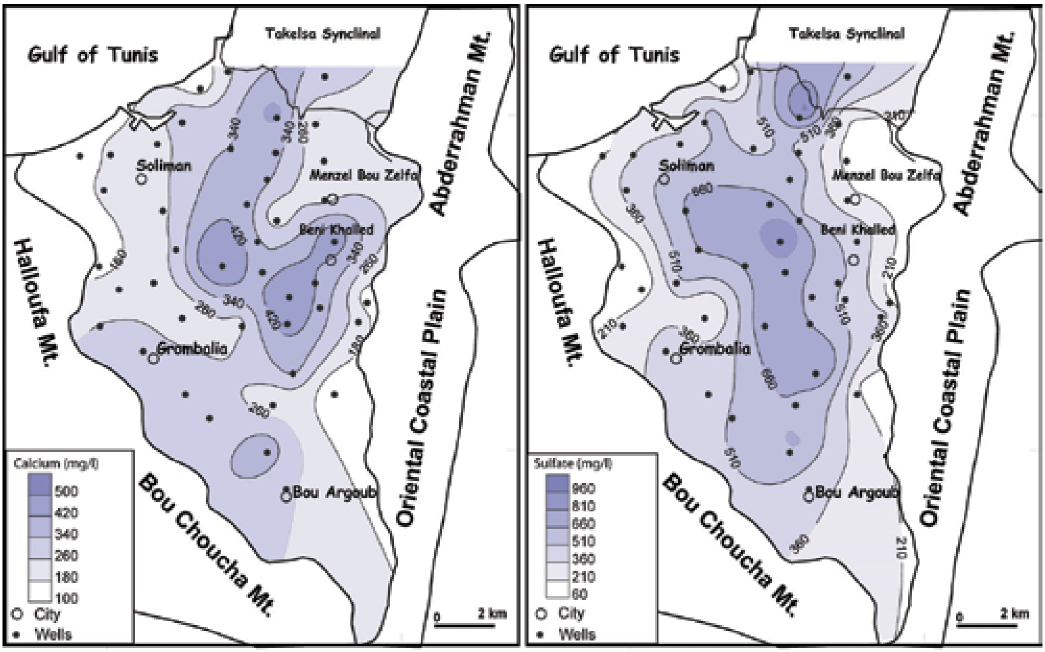

Fig. 15. Spatial distribution of Ca and SO_4

the dissolution gypsum minerals. However, some other samples display a depletion of Na *vs.* Cl that corresponds to an enrichment of Ca *vs.* SO_4, suggesting further modification by cation exchange process according to the reaction:

$$\text{Ca-Clay(s)} + 2Na^{+} \rightarrow Na_2\text{-Clay(s)} + Ca^{2+}$$

During this process, Na^{+} in the solution is exchanged with Ca^{2+} in the sediments. Moreover, the referred exchange is confirmed through the two Indices of Base Exchange (IBE), namely the Chloro-alkaline indices (CAI 1 and CAI 2) (Schoeller, 1965; Garcia et al., 2001).

$$\text{CAI 1} = Cl - \frac{Na + K}{Cl}$$

$$\text{CAI 2} = Cl - \frac{Na + K}{SO} + HCO_3 + CO_3 + NO_3$$

When there is an exchange between Na^{+} or K^{+} with Ca^{2+} or Mg^{2+} in the groundwater, both the above mentioned indices will be positive and if there is a reverse cation exchange prevalent then both these indices will be negative (Schoeller, 1965). All groundwater samples have positive IBE, indicating that cation exchange process became dominant in the study area and seems to occur along the groundwater flow path.

6. Conclusion

The present examination offers new, constructive, data for assessing the groundwater quality state of the Grombalia shallow aquifer, one of the most important water reservoirs in the Cap Bon peninsula. The results of this investigation lend support to the presence of both natural and anthropogenic processes that contribute to the groundwaters salinisation and may result in concentrations locally exceeding recommended limits. Elevated concentrations of NO_3, Br and Sr are ascribed to anthropogenic processes such as (i) the return flow of irrigated water enhanced by the flood irrigation practices, over-fertilization and pesticides leached downward. (ii) The intensive irrigation by the treated waste water; and (iii) the rejection of industrial non-treated waste waters in the drainage network. Furthermore, with increased water-rock interaction, the Grombalia shallow groundwaters naturally become more mineralized.

7. Acknowledgement

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12

Effects of Wastewater Treatment Plant on Water Column and Sediment Quality in Izmir Bay (Eastearn Aegean Sea)

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1. Introduction

Economic and social consequences of damage to the marine environment are becoming increasingly evident. Unless seas and oceans are carefully protected, their economic potential can not be sustainable. The marine environment is one of humanity's most precious assets. Oceans and seas cover 71% of the earth's surface and are the greatest sources of biodiversity, containing 90% of the biosphere. Marine ecosystems play a key role in climate and weather patterns. They also contribute to economic prosperity, social well-being and quality of life and are literally a source of survival for coastal communities. However, this environment is under intense pressure. The pace of degradation of biodiversity and habitats; the level of contamination by dangerous substances and the emerging consequences of climate change are some of the most visible warning signals (Environment for Europeans 2005). Only recently marine eutrophication is being regarded as pollution, particularly in near shore environments where more often low water transparency, oxygen depletion and algal blooms occur. Nutrient concentrations in sea water and sediment increase remarkably going from offshore to inshore, due to the proximity of terrestrial and domestic inputs and to the increase of biotic and abiotic processes strictly related to the progressive decrease of water depth.

The Bay of Izmir is in a state of pollution centre in Turkish Aegean coast region in respect of aesthetic and welfare where pollution increased in the course of time from what it used to be in 1960s. The most important factors of this current status are; domestic wastes of more than 3 million people; industrial wastes from 1,500 factories; wastewater discharge during maritime transportation and shipyard services filling materials arisen from the recreation of seaside alluvions carried with rivers and valleys. Izmir Bay is surrounded by major agricultural plateau. Menemen plateau in the North–North West of Izmir is one of the most important production fields where agricultural irrigation is utilized. The Bay is also influenced by the pollution caused by the agricultural activities in the Gediz River water shed and erosion of a large area by Gediz River.

The bay of Izmir, which is the biggest harbour on the Aegean Sea, is of economical importance for Izmir, the third largest city in Turkey. The Bay is divided into inner, middle and outer bays in terms of topographical and hydrographical characteristics. The inner bay is considerably small in area (57 km^2) and shallow in depth (max. 15 m). It had received the majority of domestic and industrial wastewaters before the construction of wastewater treatment plants. This section of the bay still receives some inflow of fresh water from several creeks which are mostly polluted by industrial wastewaters.

Because of limited water exchange with the Outer Bay and Aegean Sea, pollution of the Inner Bay had reached unacceptable levels. Eutrophication of the Inner and the Middle Bay had started and spread progressively to the outer part of the Bay. Red-tide occurrence was reported to have increase in frequency in last decade (Sunlu et al. 2007). For this reason Izmir Municipality decided to construct Izmir Big Channel WasteWater Project in 1969. However, wastewater treatment plant construction completed in 2002. At the end of the plant construction, the pollutant levels of the Inner Bay water decreased slowly and recovery period has begun (Kaymakci et al. 2000). This is why, the pollutant levels of the Inner Bay water decreased slowly.

The aim of this research is to determine the effects of “Izmir Big Channel Wastewater Treatment Project” to the sediment quality and water column of Izmir Bay. For this purpose, seawater samples and sediment samples were collected from three stations which are located in the middle and inner parts of the Izmir Bay. The water samples were collected as a weekly and sediment samples as monthly intervals during 2003.

2. Materials and methods

2.1 Location and sampling

In this study, three stations were chosen for sampling, two in the inner and one in the middle part of the Izmir Bay (Figure 1). Station 1 is near Izmir Harbor (Inner Bay) 38°27′17′′N-27°09′ 37′′E. Station 2 is offshore of the Karsiyaka Yacht Club (Inner Bay) 38°26′86′′N-27°06′56′′E and Station 3 is offshore of the Wastewater Treatment Plant (Cigli, Middle Bay) 38°25′47′′N-27°00′05′′E.

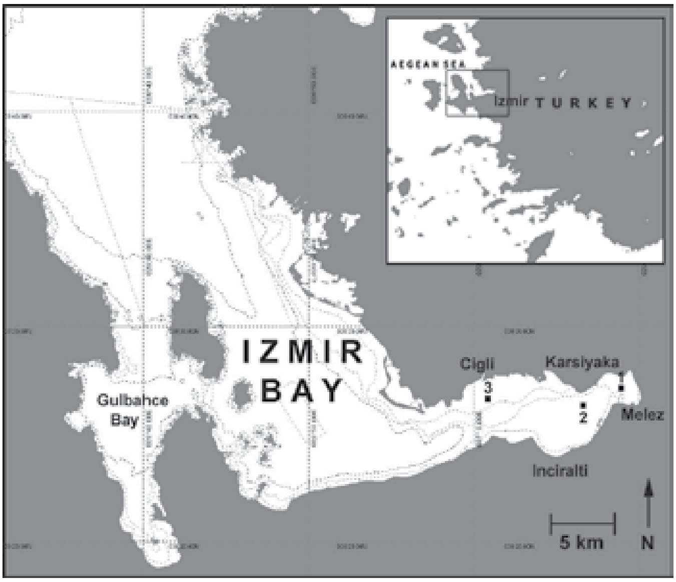


Fig. 1. Map of Izmir Bay and the sampling stations

The sampling points are shown in Fig. 1. The first sampling station is located in the area of the outflows of the Melez stream and consequently receives agricultural, domestic and industrial discharges carried on by it. This station is also influenced by the harbour activities of İzmir. The second station in Karşıyaka, shows characteristics of mixture of ST 3 and ST 1 according to current system of the İzmir Bay. The last station in Cigli where the physical and biological waste water treatment plant exists is affected by the current system of the bay. It was particularly chosen to better understand effects of this plant on İzmir Bay.

Physico-chemical environmental parameters, nutrients and some general biological parameters, were measured weekly during one year period. All these parameters were measured at different depths of the three selected sampling stations.

For this study water samples were collected using a peristaltic pump and screened 280 μ mesh to remove macrozooplankton. Polycarbonate bottles of 20 L capacity were filled with sea water and moved to the laboratory.

2.2 In situ measurements

Seawater temperature was recorded by an electronic thermometer with a sensitivity of ±0.1 °C. The pH of the samples were also measured on-site using a pH-meter (Hanna Ins.). Likewise, the dissolved oxygen concentration (DO) was measured with a portable dissolved oxygen-meter (YSI, Model 55).

2.3 Analytical measurements

The salinity of the seawater was determined by the Harvey method. The samples collected from the three different stations and different water depths were kept in 1 L polyethylene bottles and analyzed for nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), silicate and Reactive Phosphate (RP) using the methods by Strickland and Parsons 1972; Wood 1975; Parsons et al. 1984.

For chlorophyll a and phaeopigments, given amount of surface seawater was filtered through GF/C filterpaper using the Milipore Filtration system. The analyses were carried using a UVD spektrophotometer (Bosch-Lomb Spectronic 21) according to the method by Strickland and Parsons (1972). Particulate organic carbon (POC) analyses were carried out using wet oxidation method and spectrophotometry (Strickland and Parsons 1972; Parsons et al. 1984).

The detection limits and precision of methods used were given in Table 1.

PARAMETER	**PRECISION**	**DETECTION LIMITS**
NO_3^-	± 0.2 µg atN/L (1 µg atN/L at 1.7 cm. cell)	0.1-45 µg at/L
NO_2^-	± 0.2 µg atN/L (1 µg atN/L at 1.7 cm. cell)	0.1-2.5 µg at/L
NH_4^+	± 0.7 µg atN/L (10 µg atN/L at 1.7 cm. cell)	0.2-10 µg at/L
Reactive Phosphate (RP)	± 0.03 µg atP/L (3 µg atN/L at 1.7 cm. cell)	0.05-5 µg at/L
Si	0,18 µg at/L (~4 µg at/L); ± 9 (~150 µg at/L)	0,26-400 µg at/L
Salinity	± 0.05 psu	
Chl a	± 0.2 µg atN/L	0.2-50 µg Chl a,b,c /L
Dissolved Oxygen (DO)	± 0.3 mg/l (± 0.2 °C)	

Table 1. The detection limits and precision of methods used.

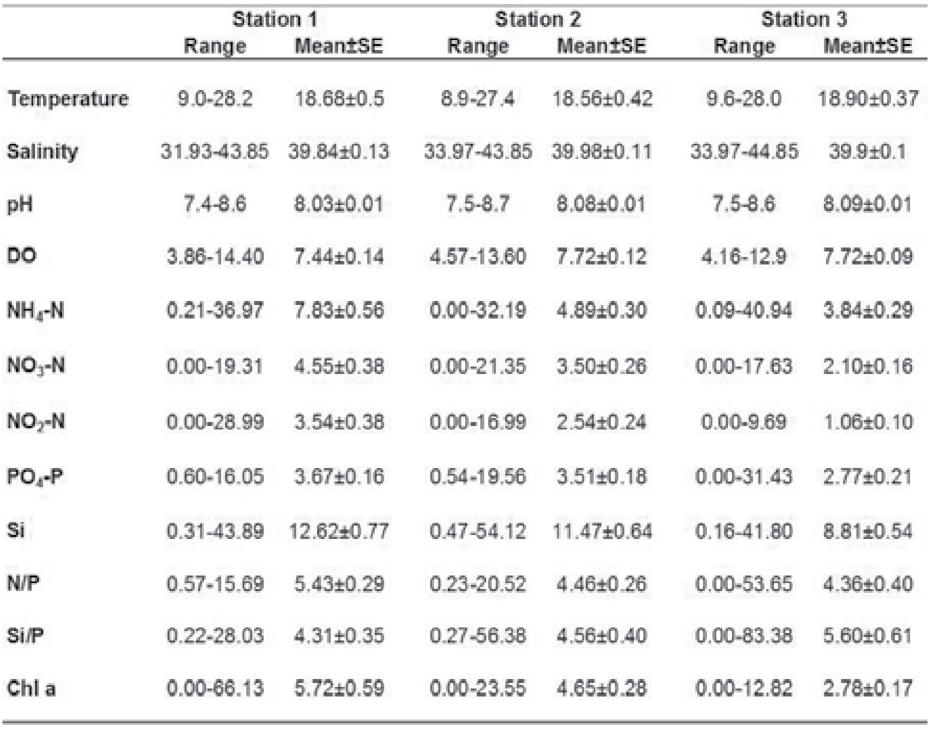
Sediment samples were collected from these three stations on a monthly basis between January and 2003 December 2003. Sediment samples were collected using Van-Veen Grap. Chlorophyll Degradation Products (CDP) were analyzed through acetone extraction and spectrophotometry (Lorenzen 1971). Organic carbon values were determined according to Modified Wakley-Black Titration Method (Gaudette et al. 1974).

3. Results

3.1 Water column

In this study, some environmentally important parameters and nutrients were measured weekly during one year period at different depths of 3 selected sampling stations in Izmir Bay.

Table 2 gives the minimum, maximum and average (± standart errors) values of the physico-chemical parameters related to the water samples from the Izmir Bay.



	Station 1		Station 2		Station 3	
	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE
Temperature	9.0-28.2	18.68±0.5	8.9-27.4	18.56±0.42	9.6-28.0	18.90±0.37
Salinity	31.93-43.85	39.84±0.13	33.97-43.85	39.98±0.11	33.97-44.85	39.9±0.1
pH	7.4-8.6	8.03±0.01	7.5-8.7	8.08±0.01	7.5-8.6	8.09±0.01
DO	3.86-14.40	7.44±0.14	4.57-13.60	7.72±0.12	4.16-12.9	7.72±0.09
NH_4-N	0.21-36.97	7.83±0.56	0.00-32.19	4.89±0.30	0.09-40.94	3.84±0.29
NO_3-N	0.00-19.31	4.55±0.38	0.00-21.35	3.50±0.26	0.00-17.63	2.10±0.16
NO_2-N	0.00-28.99	3.54±0.38	0.00-16.99	2.54±0.24	0.00-9.69	1.06±0.10
PO_4-P	0.60-16.05	3.67±0.16	0.54-19.56	3.51±0.18	0.00-31.43	2.77±0.21
Si	0.31-43.89	12.62±0.77	0.47-54.12	11.47±0.64	0.16-41.80	8.81±0.54
N/P	0.57-15.69	5.43±0.29	0.23-20.52	4.46±0.26	0.00-53.65	4.36±0.40
Si/P	0.22-28.03	4.31±0.35	0.27-56.38	4.56±0.40	0.00-83.38	5.60±0.61
Chl a	0.00-66.13	5.72±0.59	0.00-23.55	4.65±0.28	0.00-12.82	2.78±0.17

Table 2. The minimum, maximum, average and standart errors of the physico-chemical parameters related to the water samples from the Izmir Bay. Temperature (^{o}C), salinity (%o), DO (mg/l), NH_4^+-N NO_3^- -N, NO_2^--N, PO_4^{-3}-P, Si (µM), Chl a(µg/l).

3.1.1 Physico-chemical parameters

Rainfall, evoporation, streams and wastewater discharge effect variations of salinity in the bay of İzmir. As a result of rain in winter salinity has decreased. In addition salinity began to increase in during spring months suggest that water discharge out of the treatment plant left the bay along the northern shores.

Considerable increases in dissolved oxygen concentrations were observed in the first week of january, the second of march, the third of april, the first of may and the third of june. Although fluctuations were found between january and july, there was a general decrease in the values concerned, which seems consistent with the general increase found in water temperature, suggesting that dissolution of gases in water diminishes based on temperatural increase. A very slow increase was observed in dissolved oxygene concentrations from early july to the end of the year. Dissolved oxygen concentrations at ST 1 and ST 2 were found to be below the oxygen saturation.

Increases seen in pH, one of physico-chemical environmental parameters between the first week and 4th week of May were associated with those in chlorophyll a values, suggesting a decrease in inorganic carbon induced by photosynthesis, which is believed to have been caused by any indirect biological or chemical phenomenon. While significant decreases in pH appeared in mid of august, end of september and november, the general tendency to diminish in pH seems consistent with drop of dissolved oxygen saturation.

3.1.2 Nutrients

One of parameter groups quite influencing lower trophic levels in aquatic ecosystems is nutrients. Accordingly, variable values of nitrogen forms, orthophosphate and silicate based on time and depth at the three stations chosen in Izmir bay are as follows.

When nutrients and chlorophyll-a concentrations were compared to the studies carried out before the construction of Wastewater Treatment Plant (WWTP), significant decreases were observed for the nutrients (Table 6), but chlorophyll-a concentrations were higher than the values determined after WWTP by Kukrer and Aydin (2006). This situation points out the role of primary production on reduction of nutrient concentration; and thus, it is thought that this reduction transformed into the phytoplankton biomass (Kukrer, 2009).

A similarity in the spatio-temporal distributions of NH_4–N and NO_2 –N were observed at all stations. Bizsel and Uslu (2000) explain this similarity with nitrification process: NH_4–N rapidly transforms into NO_2 –N but transformation of NO_2 –N to NO_3–N is a slower process (Ozkan *et al.*, 2008). Hence, NH_4–N and NO_2 –N had similar trend in this study. NO_2 –N values were lower than NO_3–N over the sampling period. Morris (1980) was reported that this situation was normal and NO_2 –N accumulates distinguishable under low DO condition (Kukrer, 2009).

Koray *et al.* (1992) emphasized that a big part of total nitrogen in the polluted Izmir Bay was the ammonium from industrial and domestic wastes. Contrary, in this study nitrate have the biggest share in total nitrogen concentration due to the wastewater treatment plant which reduces ammonium inputs. Additionally, ammonium concentration is kept under control by phytoplankton over a year. In spite of this progress, the ammonium enrichment continues owing to the creeks and sediment which have high ammonium concentration (Ozkan *et al.*, 2008).

During the phytoplankton bloom period (January-August), NH_4–N, [$Si(OH)_4$-Si] and o.PO_4-P concentrations were lower than the values in Autumn. While inverse trend between NH_4–N and chlorophyll-*a* shows consumption of ammonium by phytoplankton, a similar relationship could not be observed between NO_3–N and chlorophyll-*a* (Kukrer, 2009).

The opposite trend between NH_4–N and chlorophyll-*a* showed the consumption of ammonium by phytoplankton, but an expected negative correlation between NO_3–N and chlorophyll-*a* could not be found. It can be explained that NH_4–N could block the uptake of NO_3–N and/or NH_4–N might be preferred by phytoplankton (Morris, 1980).

The study in which data was processed into Principal Component Analysis (PCA) aimed at determining contributions of the measured variables to total variance with different analysis for each station. All variables were defined as four major components at ST 1, explaining for 64.5% of total variance. Thus, temperature, phosphate, pH and phaeopigment account for 22.1 % of the variations involved. 18.4 % of them is to a great extent governed by DO, chl a, salinity and nitrite. 12.6 % is mostly controlled by silicate and ammonium whereas 11.3 % generally by nitrate (Table 3).

ST 1	*Component*	*Component*	*Component*	*Component*
	1	2	3	4
Phaopigment	**0,332992**	0,00587052	0,0782505	-0,345222
Temperature	0,148211	**-0,422099**	-0,15314	-0,234076
Salinity	**0,574924**	-0,113647	-0,0567523	-0,00371232
pH	**0,381094**	0,334592	-0,0951191	0,0634268
PO_4	**0,472503**	-0,0803457	0,0848547	0,368648
NO_3	-0,166612	-0,214467	-0,196831	**0,691634**
NO_2	-0,106301	**-0,364725**	-0,157476	0,155942
NH_4	0,0223274	0,240198	**0,656172**	0,133631
SiO_4	-0,0478669	-0,198481	**0,617408**	0,186501
DO	-0,292287	**0,478749**	-0,134424	-0,066133
Chl -*a*	0,20209	**0,430308**	-0,239983	0,355532

Table 3. Component Weights of ST 1

All variables of PCA made for ST2 were defined as four major components at ST 1 which explained for 62% of total variance. 21% of it often depends on temperature, phosphate and DO whereas its 17.4 % is mostly controlled by nitrite, nitrate, pH. 14% of variance is found to be accounted for by ammonium and silicate while its 9.6% is to a great measure under the control of phaeopigment, salinity and chl a (Table 4).

ST 2	*Component*	*Component*	*Component*	*Component*
	1	2	3	4
Phaopigment	0,190085	0,033317	-0,337102	**0,416977**
Temperature	**0,612123**	-0,0620875	0,00251255	0,050634
Salinity	0,175982	-0,389765	-0,0522611	**0,45086**
pH	0,350358	**0,409077**	-0,0404681	-0,241227
PO_4	**0,443203**	0,0770914	-0,0509096	-0,124404
NO_3	-0,120681	**-0,436475**	-0,160533	-0,282205
NO_2	-0,0501546	**-0,528066**	-0,152599	0,0743837
NH_4	-0,0090085	0,17262	**0,513087**	0,382987
SiO_4	0,0249009	-0,156039	**0,622841**	0,218553
DO	**-0,469988**	0,328992	-0,165754	0,194231
Chl -*a*	0,0188411	0,195156	-0,389454	**0,478833**

Table 4. Component Weights of ST 2

All variables were described as four major components at ST 3 which explained for 61.2% of total variance. 25.2 % of total variance is generally explained by temperature, phosphate, oxygen and phaeopigment Nitrate is seen to be responsible for 14.9 % of it whereas its 11.8% is basically governed by salinity, chlorophyll a and nitrite. On the other hand 9.3 % of total variation is mostly controlled by silicate and ammonium (Table 5).

ST 3	*Component*	*Component*	*Component*	*Component*
	1	2	3	4
Phaopigment	**0,309441**	-0,115255	-0,118439	0,00891844
Temperature	**0,478778**	0,368824	-0,00879898	0,140916
Salinity	-0,024596	0,365174	**-0,519479**	0,253368
pH	**0,33159**	0,186109	0,321044	-0,0842017
PO_4	**0,437231**	-0,22811	0,0675536	0,134745
NO_3	0,200952	**-0,554785**	-0,129602	0,162289
NO_2	0,0991039	-0,401237	**-0,46215**	0,29997
NH_4	0,285814	-0,197389	0,0871168	**-0,468515**
SiO_4	0,244234	-0,0379852	-0,22798	**-0,566497**
DO	**-0,383154**	-0,345061	0,258506	-0,0662661
Chl -*a*	0,186924	-0,0491215	**0,501633**	0,479047

Table 5. Component Weights of ST 3

Table 6 shows minimum and maximum values of nutrients and *Chl a* in some previous studies which were carried out in the different parts of the Izmir bay. Izmir Wastewater Treatment Plant Construction was completed in the 2002. It works on the principle of nitrogen and phosphorus treatment technology with activated sludge. Previous studies indicated that the concentration of TNO_x-N has been reduced during after wastewater activated sludge technology plant except sudden discharge, while reactive phosphate concentrations were increased in the Bay. In the Middle and Inner Parts of the Bay Chlorophyll *a* concentration has been gradually reduced after treatment.

In conclusion, we are of the opinion that it would be of great use to develop and plan further similar studies periodically and for the long run considering that they could shed light on precautions to be taken in terms of both environmental and public health.

The changes in the state variables of ecological model for İzmir Bay before and after the sewage treatment has been given by Büyükışık et al., 1997 (Fig.2 and 3). They reported that average light intensities in water column would be recovered in a year if the treatment plant begins to work. Indeed, after one year from starting of sewage treatment (2003), the observation in recovery of the average light intensities in water column consistent with the model outputs in case of treatment.

But some changes in temporal variations of phytoplankton biomass has been observed (Fig.4). Some exceptional blooms has taken place in mid-winter, early summer and autumn. Model does not includes the kinetic parameters of *Ditylum brightwellii* (in winter) and *Rhizosolenia setigera* (in summer).

These two species are relatively large sized phytoplankton and they contributed greatly to the total phytoplankton carbon and POC values.

Specially some members of genus Rhizosolenia can change their cellular density, sink deeper, uptake and storage the nutrients and go on their growth.

Locations	Period	NO_3(µM)	NO_2(µM)	NH_4(µM)	Si(µM)	RP(µM)	Chl a(µg l^{-1})	Reference
Inner part of Izmir Bay	1993-1994	BDL-3,04*	BDL-4,65*	0,12-468*	-	0,36-49	BDL-189	Bizsel, Uslu,2000
Middle part of Izmir Bay	1993-1994	BDL-3,49	BDL-3,57	BDL-44	-	0,06-3,79	0,5-62	Bizsel, Uslu,2000
Outer part of Izmir Bay	1993-1994	BDL-4,91	BDL-0,16	BDL-11,11	-	BDL-6,42	BDL-2,95	Bizsel, Uslu,2000
Inner part of Izmir Bay	1993-1994	BDL -3,11	BDL -4,65	BDL -468	-	0,18-49	BDL -189***	Bizsel, Uslu,2000
Candarlş Bay (Aegean Sea)	1994-1995	0,001-0,31	BDL-0,1	0,42-2,38	27,74-63,19	BDL-0,48	BDL-1,13	Aksu et.al. 2010
Middle-Inner part of Izmir Bay	1996-1998	0,13-27	0,01-18	0,10-21	0,50-39	0,01-10	0,10-26	Kucuksezgin, et. al. 2006
Middle-Inner part of Izmir Bay	2000	0,15-18	0,02-12	0,13-34	0,43-20	0,13-3,8	0,46-18	Kucuksezgin, et. al. 2006
Middle-Inner part of Izmir Bay	2001	0,29-16	0,02-4,3	0,11-50	1,2-18	0,14-2,9	0,38-7,8	Kucuksezgin, et. al. 2006
Middle-Inner part of Izmir Bay	2002	0,26-6,7	0,01-6,1	0,10-6,7	1,0-26	0,14-4,4	0,13-3,7	Colak-Sabancş, Koray, 2001
Gerence Bay (Aegean Sea)	2002	0,04-2,19	BDL-2,51	BDL-3,53	-	BDL-2,82	BDL-0,320	Aydşn Gençay, Büyükşşk, 2006
Middle-Inner part of Izmir Bay	2003	0,12-8,6	0,01-1,0	0,12-2,4	2,6-32	0,32-4,5	0,24-2,6	Colak-Sabancş, Koray, 2001
Inner part of Izmir Bay	2007-2008	1,54-11,77	0,00-3,51	0,23-22,28	1,99-41,94	0,00-5,96	5,03-30,26	Kukrer, 2009
This Study	2003	BDL-21,35	BDL-28,99	BDL-40,94	0,16-54,12	BDL-31,43	BDL-66,13	This Study

* Min-Max;

** Average value;

*** Data from (32);

BDL: Below Detection Limits

Table 6. Minimum and maximum concentrations of nutrient and chlorophyll-a in Izmir Bay and Aegean Sea from different studies

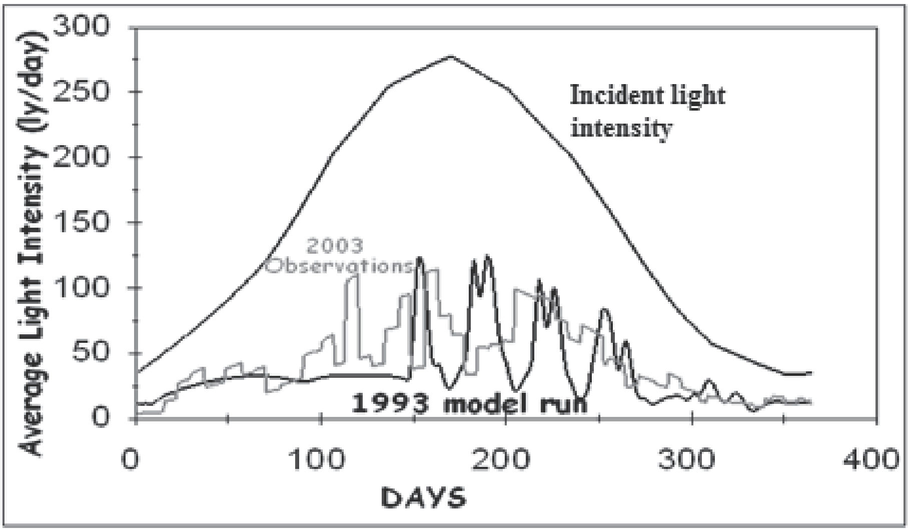


Fig. 2. Temporal changes of the average water column light intensities obtained from model in 1993 (Black curve, Büyükışık et al 1997) and from chl-*a* values in 2003 (gray lines, Sunlu et.al, 2007). The black curve at top represents the temporal changes in incoming sub-surface light intensities (Büyükışık et al 1997).

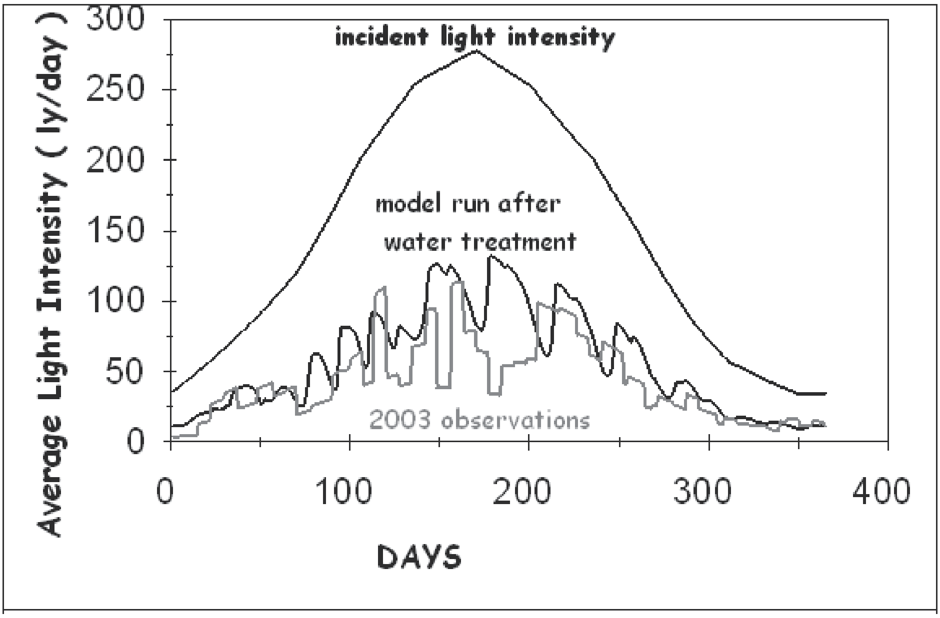


Fig. 3. Temporal changes of the average light intensities obtained from model in case of 90% nutrient treatment (black curve, Büyükışık et al 1997) and from chl-*a* values in 2003(gray lines, Sunlu et al, 2007). The black curve at top represents the temporal changes in incoming sub-surface light intensities (Büyükışık et al 1997).

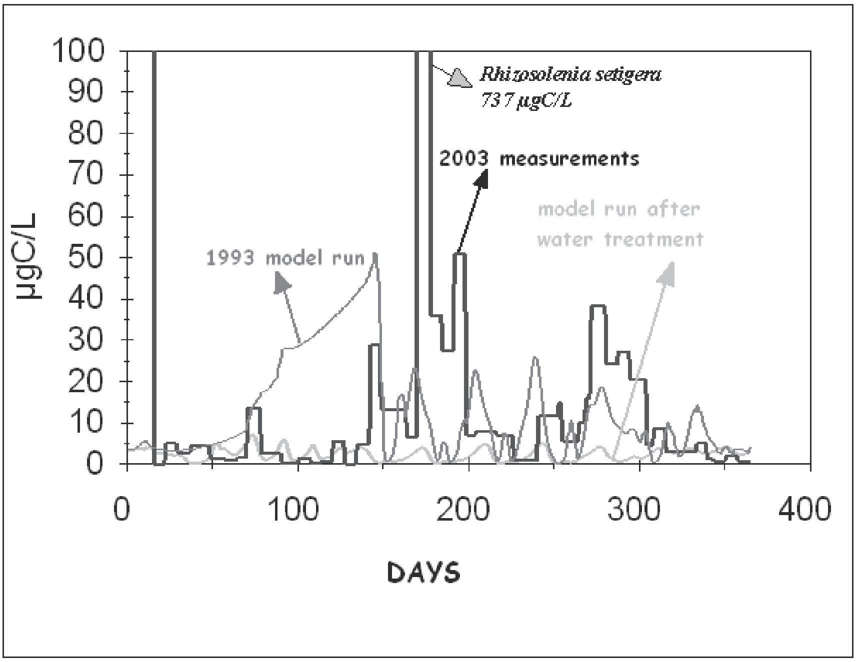


Fig. 4. Temporal changes of the phytoplankton biomass obtained from model in case of 90% efficiently treatment (light gray curve, Büyükışık et al 1997). The dark gray curve represents the model run outs in 1993 (moving average, Büyükışık et al 1997). Black column in graph represents the measurements in 2003 from biomass calculates two microscopic examinations (Sunlu et al, 2007).

3.2 Sediment

Values measured at stations ranged between; 0.09–9.32 µg/L for phaeopigment, 0.05–1.91 mg/L for particulate organic carbon in sea waters, 11.88–100.29 µg/g for chlorophyll degradation products and 1.12–5.39% for organic carbon in sediment samples. In conclusion, it was found that grazing activity explained carbon variations in sediment at station 2, but at station 1 and station 3 carbon variations in sediment were not related to autochthonous biological processes.

3.2.1 Organic carbon in sediment

Organic carbon values at station 1 ranged from 2.63 to 3.39%. Average concentration was 3.03%. Minimum, maximum and average organic carbon values at station 2 were 1.73, 5.39 and 4.33% respectively. Organic carbon values at station 3 ranged from 1.12 to 2.41%. Average concentration was 1.58% (Fig. 5). Previous carbon contents in the sediment samples from the different regions of Aegean Sea were given in Table 7.

3.2.2 Chlorophyll degradation products in sediment (CDP)

Chlorophyll degradation products in sediment at station 1 ranged from 50.79 to 90.66 µg/g and average value was found 62.62 µg/g. At station 2 average CDP value was 81.39 µg/g. Minimum and maximum values were measured as 41.58–100.29 µg/g respectively. CDP

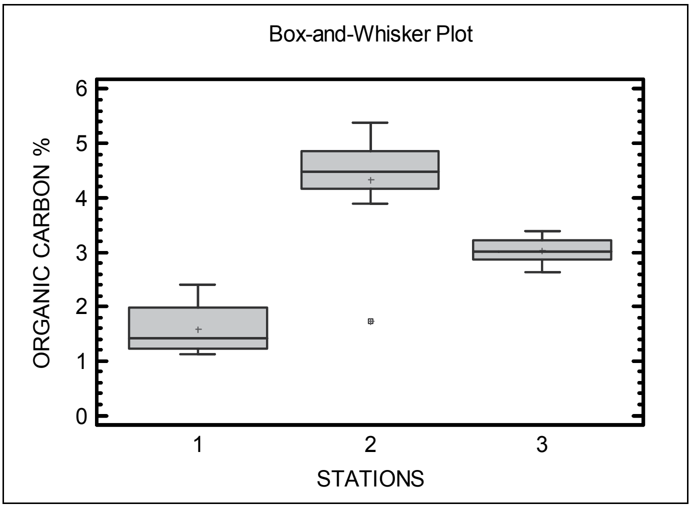


Fig. 5. Box and whisker plot of Organic carbon (%) values at all sampling stations.

concentrations at station 3 ranged from 11.88 to 52.12 µg/g. Annual mean was 34.44 µg/g(Fig. 6). When each three region was discussed separately, at the Station 2, algal sedimentation and/or mesozooplankton grazing explain variations of carbon in the the sediment samples (r=0.7879 p=0.0023). According to statictical analyses of C sed/CDP for each region, variations of CDP in sediment seems independent from carbon in sediment variations for station 1 and station 3 in sequence (r=0.339, r=0.206). Melez, Manda and Arap Rivers discharge their waters rich in organic mater around station 1 (Turkman 1981). At station 3, during the year CDP concentrations were at the lowest value and it can be explained by background carbon levels that mask carbon variations which is caused by algae (< %2). Besides, the output of the wastewater treatment plant is close to the station 3 and it constitutes crucial silicate source. Diatoms consist of skeleton with silica are known as having five times lower carbon content than Dinoflagellates (Hitchcock 1982 in Smayda 1997). That situation can explain that during the year phytoplankton community has lower carbon content. Even if export production to sediment increases relatively low productivity and low carbon content in water column can cause a similar situation in diatom dominated marine environments. By using overall data in Inner and Middle Izmir Bay, chlorophyll degradation products in sediment versus carbon values were plotted. A good linear relationship between CDP and carbon was obtained (r2=0.771, p=0.000):

$$[\text{Carbon}]_{\text{sed}}=0.2077+0.0466*[\text{CDP}]_{\text{sed}}$$

A general equation was found for predicting the Izmir Inner Bay's CDP and organic carbon values in sediment. It was found that there are no significant differences in sediment carbon values depending on time but spatial variations related to sampling stations are more evident. When spatial scale is widened, CDP variations explained 77% of carbon variations in the sediment for overall data. Approximately 23% of these variations were originated from allocthonous sources.

At station 3, it is possible that grazing on diatoms and/or mixotrophy in dinoflagellates are dominant on certain onths of the year. Consequently, it is not possible to explain variations of the carbon in sediment with the pigment contents of sediment. Station 2 has highest carbon and CDP values and also has a relationship between CDP and organic carbon content. This situation can be explained by the fact that station 2 is relatively away from external sources and has high biological activity (Sunlu et al. 2007). At station 1, however, relation is weak despite higher carbon and CDP values than at station 3. Contribution of external carbon sources as rivers may play important role on this weak correlation.

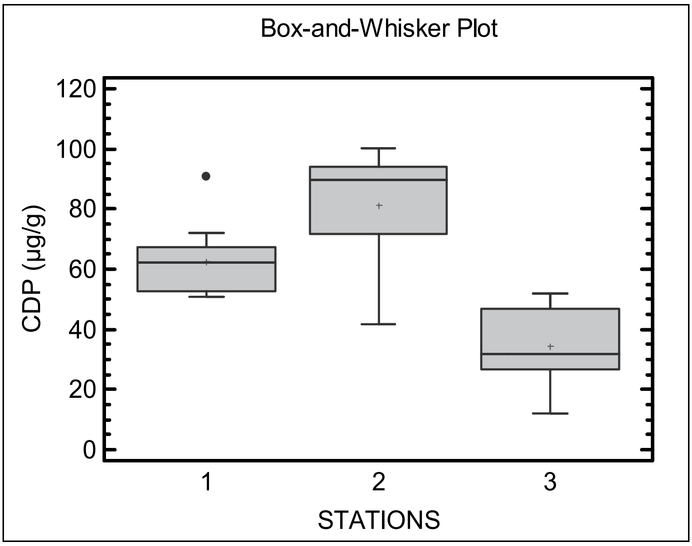


Fig. 6. Box and whisker plot of CDP (µg/g dry sediment) values at all sampling stations.

Locations	Carbon in Sediment (%)	Reference
Middle part of Izmir Bay	0.87-1.60	Yaramaz et. al. 1992
Inner part of Izmir Bay	0.57-3.42	Yaramaz et. al. 1991
Izmir Bay	11.4	Anonymous, 1992
Izmir Bay	2.0-7.0	Anonymous, 1997
Gulluk Bay (Southern Aegean Sea)	0.1-4.5	Egemen et. al. 1999
Gulluk Bay (Southern Aegean Sea)	1.07-2.13	Atılgan, 1997
Urla (Middle part of Izmir Bay)	1.25-2.1	Sunlu et. al. 1999
Pariakos Bay (Greece)	0.15-11.01	Varnavas and Ferentionos, 1982
Evoikos Bay (Greece)	1.2	Scoullos and Dassennakis, 1982
Evoikos Bay (Greece)	0.66-2.4	Angelidis et. al. 1980
Southern Turkish Aegean Sea	1.3-13.1	Aydın and Sunlu, 2005
Northern Turkish Aegean Sea	0.35-15.63	Sunlu et. al. 2005
Middle part of Izmir Bay	1.12-5.39	This Study

Table 7. Previous carbon contents in the sediment samples from the different regions of Aegean Sea.

4. Conclusion

When our mean results were compared with those obtained before Izmir wastewater treatment plant was operating, concentrations of chlorophyll a and nitrogen forms declined while it was not the case for orthophosphate.

The fact that the processes affecting Reactive Phosphate (RP) and TIN occur at different times indicates important differentiations in the temporal variations of these two nutrients in the Inner Bay. From the distribution of the nutrients and their percentages, important evidence regarding the process have been gathered. These processes:

- Inflow with the creeks is especially evident during rainfall and there is a big increase in Si and Nitrogen forms.
- Rapid decreases of freshwater inflows from rainfall based on current global warming tend to restrict Si and N inflows. Water outflow treated from treatment plant is another source of nutrient with N/P ratios being about <=2. RP induced by water from treatment plant thus contributes to RP reserves in Inner Bay.
- The winds, although increasing fresh water inflow and water column, frequently carry the deep water to the surface. This shows that the Inner Bay is often subject to a deep-water-based nutrient enrichment.

The phytoplankton blooms caused by the inflow of nutrients to the Inner Bay in turn result in the intake of nutrients by the phytoplanktons (especially diatoms) which are then exported to the deep waters and constitute the fuel for future phytoplankton blooms. Thus, the horizontal exportation of the nutrients out of the Inner Bay remains limited. It is only due to the winds that the wastewaters flow outwards from time to time.

Because total renewal of Inner Bay water by the current system takes about ten days, nutrient load provided by various sources in the area is most important reason for overgrowth of phytoplanktons observed in the Izmir Bay.

Silicate is essential for the diatoms to compete effectively with dynophylagellates and plays an important role in the increase in species in the bay and this nutrient, coming with the rainfall from the shore in non-point sources and point sources (i.e. creek, river), is of great importance for the Inner Bay.

We believe that unless the nutrient levels in the rivers are decreased, the Bay will continue its current state for a long time. Although a decrease has been observed in the nitrogen nutrients after the start of the wastewater treatment plant, former studies have shown that the phosphate concentrations have not changed and that the plant has been ineffective regarding this subject. The effective treatment of phosphate will be an important precaution against the new strategy that the phytoplankton might take up against the decreasing TIN.

The reason for this was that 2– 10 years elapsed between the two studies and the treatment facility begun to work in full capacity in 2002. On the other hand; carbon contents in the sediment samples of our study are considerably lower compared with the values obtained in a large scale previous research carried out by different regions around Aegean Sea.

General sediment texture of Izmir Bay was studied by Duman et al. (2004). Average sediment particle size was reported to be 4–8 ф and sediment texture to be sandy-silt. In Izmir Bay sorting coefficient indicates very poorly sorted deposits (SD=2–3). Prevailing wind direction in inner part of Izmir Bay was noted as Western and it has been reported that deep flow was toward to East and surface flow toward to West. Most of organic material remains in the silt near the pollution source and the correlation between grain size fractions and organic carbon was found to be highest in silt (Duman et al. 2004). One sediment component, vermiculite was found in the inner part of Izmir Bay at a rate of 3–11% and its main source was from Melez River (near station 1). Caolinit was found at a rate of 8–12% with neogen sediments coming from the rocks around the Bay (Aksu et al. 1998). Percentage of organic carbon was reported to be between 0.40 and 5.39 by Duman et al., from Izmir Bay (Duman et al. 2004). Range for these values was found to be between 1.12 and 5.39% in our study. These values were higher than previous report (Duman et al. 2004). The reason for this was that 2– 10 years elapsed between the two studies and the treatment facility begun to work in full capacity in 2002. On the other hand; carbon contents in the sediment samples of our study are considerably lower compared with the values obtained in a large scale previous research carried out by different regions around Aegean Sea (Table 7). It can be said that high carbon levels observed in inner part of Izmir Bay were from raw sewage and industrial outfalls carried by Melez River at station 1. But at station 2 and 3 high carbon levels were due to organic material formed by secondary pollution. The biggest contribution to the sediment is provided byexport production which was especially effective at station 2. A general equation was found for predicting the Izmir Inner Bay's CDP and organic carbon values in sediment. There are no significant differences in sediment carbon values depending on time but spatial variations (related to sampling stations) are more evident . In conclusion, it was found that carbon variations in sediment at station 2 (Karşıyaka, Offshore of the Yatch Club) can be explained by grazing activity, but at station 1 (Melez, Izmir Harbour) and station 3 (Cigli, Offshore of the Wastewater Treatment Plant) carbon variations in sediment could be related not only with autochthonous biological processes but also with physical processes (e.g. sweeping out of plant material by advection from the Bay). Especially wastewater treatment improves the water quality, but sediment does not respond to this treatment as fast as water column. Improvement in the quality of bottom water and sediment is the evidence of the recovery of the whole ecosystem of the Izmir Bay.

In conclusion, it was found that carbon variations in sediment at station 2 (Karşıyaka, Offshore of the Yatch Club) can be explained by grazing activity, but at station 1 (Melez, Izmir Harbour) and station 3 (Cigli, Offshore of the Wastewater Treatment Plant) carbon variations in sediment could be related not only with autochthonous biological processes but also with physical processes (e.g. sweeping out of plant material by advection from the Bay).

Especially wastewater treatment improves the water quality, but sediment does not respond to this treatment as fast as water column. Improvement in the quality of bottom water and sediment is the evidence of the recovery of the whole ecosystem of the Izmir Bay.

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13

Effects of Domestic Waste Water on Water Quality of Three Reservoirs Supplying Drinking Water in Kaduna State - Northern Nigeria

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1. Introduction

Waste water management in Nigeria does not receive the attention it deserves. Domestic waste water is discharged into streams and reservoirs that supply drinking water without any treatment (Tiseer *et al.,* 2008). Chemical substances from agricultural activities (fertilizers, pesticides and herbicides) in the catchment of reservoirs may introduce nutrients and heavy metals at concentrations higher than that which the environment can handle (WHO, 2006). Nigeria has a number of environmental regulatory laws which include: the National Environmental Standards and Regulations Enforcement Agency (Establishment) Act of 2007 (The NESREA Act), Nigerian Radioactive Waste Management Regulations 2006, Environmental Impact Assessment Act of 1992 (EIA Act), Harmful Wastes (Special Criminal Provisions etc.) Act of 1988 (Harmful Wastes Act), the National Oil Spill Detection and Response Agency (Establishment) Act 2006 (the NOSDRA Act) and Nigerian Radioactive Waste Management Regulations 2006. However, the enforcement of these regulations has not been effective (Onaruwa and Fakayode, 2002 and Adegoroye, 2008) and thus pollution of both rural and urban water sources commonly occurs. In rural areas, natural sources of drinking water, such as streams, wells and other reservoirs are usually polluted by organic substances from upstream users who use water for Agricultural activities and other domestic purposes. In urban areas, population pressure, industrial activities and agricultural activities place pollution stress on reservoirs of water (Adakole *et al.,* 2002, Fakayode, 2005 Kimura, 2005, Tiseer *et al.,* 2008). The water in these reservoirs is sometimes taken directly without any form of treatment.

Contamination of sources of water by waste alters water quality (the physical, chemical and biological characteristics).When the physical and chemical conditions of ecosystems are changed beyond their normal ranges, changes may be expected to occur in individual organisms, populations and communities of the ecosystem (Lenat *et al.*, 1980, Akin-Oriola, 2003, Kadiri, 2006). Assemblages of cyanobacteria are good indicators of eutrophic water bodies (Reynolds, 1998). Some species of cyanobacteria could contain cyanotoxins in their cells but do not release these into the water, and as such are harmful only when consumed while others release toxins directly into the water (Chorus and Batram, 1999 and WHO, 2006). They can also alter taste and odor problems, cause water discoloration, or form large mats that can intefere with boating, swimming, and fishing (Borgh, 2004). Cyanobacteria present a range of characteristics that give them clear competitive growth advantage over planktonic algae under certain environmental conditions. Such include; a requirement of low light intensity and little energy to maintain cell structure and function (Mur *et al.*, 1999); possession of gas vacuoles within their cells as a buoyancy regulation mechanism to avoid light damage in high-light environments, such as in tropic lakes or to access light in turbid or low-clarity water (Haider *et al.*, 2003). Cyanobacteria can also store phosphorus (luxury uptake), as a useful adaptation that allows continued growth under conditions of fluctuating nutrient concentrations. They are also not grazed by zooplankton, since they are not the preferred food for these aquatic organisms (Chorus and Batram, 1999).

Data on levels of aquatic pollution and its implication to human health is generally lacking for most aquatic ecosystems in Nigeria. This study was therefore designed to evaluate the impact of waste water on three reservoirs receiving varying degrees of waste water.

2. Materials and methods

2.1 Study area

The three reservoirs studied were Gimbawa reservoir in Ikara Local Govt. (Long.10^{0}6′N and Lat.8^{0}35′E), Saminaka reservoir in Lere Local Govt. (10^{o}70′N and 8^{o}75′E) and Zaria reservoir, Zaria Local Government (7^{0} 38′N and 11^{0}11′E) of Kaduna State. Kaduna State is located in the northern guinea savannah vegetative zone of Nigeria and has a tropical continental climate, with distinct wet and dry seasons. Three sampling stations were studied in each reservoir based on the diffrent activities in the catchment from May 2008 to April 2009.

2.2 Phytoplankton collection:

Phytoplankton was collected using a conical shape plankton net of 20 cm diameter with a 50 ml collection vial attached to it (Perry, 2003). Samples were collected at three sampling points in each reservoir to reflect the various activities in the catchment. Phytoplankton was identified by consulting texts by Presscott (1977) and Perry (2003).

2.3 Physico-chemical parameters

Physico-chemical parameters of water were analyzed once a month from May 2008 to April 2009. Surface water temperature was measured *in situ* using a mercury thermometer. pH and Electrical Conductivity were measured using HANNA instrument (pH/Electrical Conductivity/Temperature meter model 210). Total Hardness, Dissolved oxygen (DO), Biological Oxygen Demand (BOD), Nitrate-Nitrogen (NO_3-N) and Phosphate-phosphorus (PO_4-P) were determined by methods described by APHA (1998).

2.4 Metal analysis

Metal concentration in the water samples was determined by Atomic Absorption Spectrophometry (AAS). Water samples were digested by Nitric acid (HNO_3) digestion (as described by APHA, 1998).

3. Statistical analysis

Analysis Of Variance (ANOVA) was used to compare the means of physicochemical parameters; heavy metals concentration and abundance of phytoplankton from the different reservoirs. Pearson's correlation coefficient was used to determine the relationship between physicochemical parameteres; physicochemical parametres and phytoplakton. Shannon-Wiener diversity index was used to determine phytoplankton diversity while Simpson's Index was used to determine evenness of phytoplankton distribution.

4. Results

Mean monthly Air Temperature varied from 27.67 to 34.17^{0}C with mean ± standard error of 31.76±0.62^{0}C (Table1), for Gimbawa reservoir, whereas in Saminaka reservoir it ranged between 25^{0}C and 36.67^{0}C with mean ± SE of 30.96±0.97^{0}C. In Zaria reservoir, air temperature ranged from 26 to 35.33^{0}C mean ± SE of 29.67±0.68^{0}C(Table 1). This observed difference was however not statistically significant.

The three reservoirs had mean ± SE of Surface water temperature was 26.16±1.00^{0}C (Gimbawa), 26.19±1.07^{0}C (Saminaka) and 26.08±0.63^{0}C (Zaria) (Table 1). The differences were however, not statistically significant between months, seasons and reservoirs (P > 0.05).

	Gimbawa			**Saminaka**			**Zaria**		
	Min	**Max**	**Mean ± SE**	**Min**	**Max**	**Mean ± SE**	**Min**	**Max**	**Mean ± SE**
Air Temperature (^{0}C)	27.67	34.67	31.76 ± 0.62	25	36.67	30.96 ± 0.97	26	35.33	29.67 ± 0.68
Water Temperature (^{0}C)	20.33	31.67	26.16 ± 1.00	20	31	26.19 ± 1.07	20.67	28	26.08 ± 0.63
Secchi disc Transparency (cm)	13.67	69.67	17.67 ± 6.06	8.17	19.33	7.29 ± 2.19	13.67	47	21.48 ± 4.46
pH	6.87	8.76	7.54 ± 0.15	6.46	8.21	7.34 ± 0.15	6.42	7.9	7.31 ± 0.14
Electrical Conductivity (µS/cm)	45.1	573.33	120.50 ± 41.95	12.33	496	128.07 ±40.00	31.67	518	97.20 ±38.59
Dissolved Oxygen (Mg/L)	6.87	8.76	6.71 ± 0.39	3.52	9.1	6.16 ± 0.53	3.73	10.22	6.44 ± 0.58
BOD (Mg/L)	0.16	4.37	2.17 ± 0.41	0.37	5.57	2.60 ± 0.5	0.06	3.54	1.68 ± 0.38
Alkalinity (Mg/L)	2.87	6.7	5.05 ± 0.32	2.43	14.8	6.77 ± 1.16	2.5	5.8	4.29 ± 0.31
Hardness (Mg/L)	0.5	3.93	1.26 ± 0.26	0.43	4.53	1.46 ± 0.30	0.6	5.1	
N0$_3$-N (Mg/L)	0.03	0.19	0.12 ± 0.01	0.02	0.16	0.09 ± 0.01	0.01	0.55	0.13 ± 0.05
P0$_4$-P (Mg/L)	0.06	0.62	0.29 ± 0.06	0.06	0.76	0.39 ±0.08	0.03	0.8	0.39 ±0.08

SE = Standard Error, BOD = Biochemical Oxygen Demand, N0$_3$-N = Nitrate-Nitrogen, P0$_4$-P = Phosphate-phosphorus

Table 1. Physico-chemical characteristics of Gimbawa, Saminaka and Zaria reservoirs

Secchi Disc Transparency values in Gimbawa reservoir had the highest value of 69.67cm and lowest of 13.67cm. In Saminaka reservoir, the values ranged from 4.36 to 19.33cm, while in the Zaria reservoir it ranged from 13.67 to 47cm. The mean ± Standard Error of the reservoirs are Gimbawa: 17.67±6.06cm, Saminaka: 7.29±2.19cm and Zaria: 21.48±4.46cm (Table 1). This observed difference was statistically significant between reservoirs ($P < 0.05$) and between seasons ($P < 0.05$).

pH values in Gimbawa reservoir varied from 6.87 to 8.76. In Saminaka reservoir, the highest pH value was 8.21 and lowest was 6.46.While in Zaria reservoir, the highest pH value was 7.9 and lowest of 6.42. The mean±SE observed in the reservoirs were: Gimbawa, 7.54±0.15; Saminaka, 7.44±0.15 and Zaria, 7.31±0.14 (Table 1). The observed differences were not significant between reservoirs ($P > 0.05$) but significant between months ($P < 0.05$) and seasons ($P < 0.01$).

The mean±SE Electrical of Conductivity (EC) for Gimbawa, Saminaka and Zaria reservoirs observed were 120.50± 41.95μS/cm, 128.07± 40.00μS/cm and 97.20± 38.59μS/cm respectively (Table 1). The variation of EC was significant only between months ($P < 0.05$).

Dissolved Oxygen (DO) varied between 8.58mg/L and 3.9 mg/L in Gimbawa reservoir,. Saminaka reservoir had values ranging between 9.1mg/L to 3.52ml/L while in Zaria reservoir had range of values for DO from 3.73 mg/L to 10.22 mg/L. The mean±SE of Gimbawa, Saminaka and Zaria reservoirs observed were 6.71± 0.39 mg/L, 6.16± 0.53mg/L and 6.44 ± 0.58 respectively (Table 1). The variation of DO was significant between months and seasons ($P < 0.05$).

Biochemical Oxygen Demand (BOD) values in Gimbawa reservoir ranged from 4.37mg/L to 0.16mg/L, In Saminaka reservoir the values range from 0.37 to 5.57mg/L whereas in Zaria reservoir the values ranged from 0.06mg/L to 3.54mg/L. The mean±SE of Gimbawa, Saminaka and Zaria reservoirs observed were 2.17± 0.41 mg/L, 2.60± 0.50mg/L and 1.68 ± 0.38mg/L respectively (Table 1). The variation of BOD was significant between months and seasons ($P < 0.01$).

The mean ± SE of Alkalinity for Gimbawa, Saminaka and Zaria reservoirs observed were 5.05± 0.32 mg/L, 4.29± 0.31mg/L and 6.77 ± 1.16mg/L respectively (Table 1). The variation of Alkalinity was significant between months, reservoirs ($P < 0.05$) and between seasons ($P < 0.01$).

The mean ± SE of Hardness for Gimbawa, Saminaka and Zaria reservoirs observed were 1.26± 0.26 mg/L, 1.46± 0.30mg/L and 1.49 ± 0.36mg/L respectively (Table 1). These variations however, were only significant between months ($P < 0.05$) and not between months and seasons ($P > 0.05$).

Nitrate-nitrogen concentration for Gimbawa reservoir had a highest value of 0.19 mg/L and lowest of 0.03mg/L. Saminaka reservoir had a highest value of 0.16 mg/L and lowest of 0.02mg/L. Zaria reservoir had its highest value of 0.55 mg/L and lowest of 0.01 mg/L. The mean ± SE Nitrate-nitrogen concentration for Gimbawa, Saminaka and Zaria reservoirs observed were of 0.01 mg/L, 0.09± 0.05mg/L and 0.13 ± 0.05mg/L respectively (Table 1). These variations however, were not statistically significant between reservoirs, months and seasons ($P > 0.05$).

For phosphate-phosphorus concentration, Gimbawa had its highest value of 0.62mg/L and lowest of 0.18mg/L. Saminaka reservoir had the highest concentration of 0.76mg/L and lowest of 0.04mg/L. Zaria reservoir had its highest value of 0.8mg/L and lowest of 0.04mg/L. The mean±SE of Gimbawa, Saminaka and Zaria reservoirs observed were 0.29± 0.06 mg/L, 0.39 ± 0.08mg/L and 0.39 ± 0.08mg/L respectively (Table 1).These variations however, were only significant between months ($P < 0.01$) but not between reservoirs and seasons ($P > 0.05$).

4.1 Metal ions

The lowest concentrations of Cu, Zn, Mn, Fe and Cr were below detectable limits in the three reservoirs. The highest concentration of Cu, Zn and Cr was recorded in Zaria reservoir (0.39, 0.50 and 1.10 mg/L respectively). Gimbawa reservoir had the highest concentration of Mn (1.01mg/L) and Fe (1.14mg/L). The mean ± SE of these metals in Gimbawa, Saminaka and Zaria respectively are Cu: 0.03 ± 0.03mg/L, 0.03 ± 0.02mg/L and 0.04 ± 0.03mg/L; Zn: 0.03± 0.03 mg/L, 0.02± 0.01 mg/L and 0.04 ± 0.04 mg/L; Mn : 0.08 ± 0.08, 0.09 ± 0.06mg/L and 0.06 ± 0.06 mg/L mg/L; Fe: 0.28± 0.1 mg/L, 0.89± 0.43 mg/L and 0.51± 0.28 mg/L and Cr: 0.43± 0.07 mg/L, 0.36± 0.06 mg/L and 0.34 ± 0.08.

Concentrations of Nickel in the three reservoirs showed the highest concentrations of 1.06, 1.0 and 1.17 mg/L; and lowest concentrations of 0.17, 0.26 and 0.17 mg/L for Gimbawa, Saminaka and Zaria reservoirs respectively (Table 2). The mean ± Standard Error for the reservoirs were 0.64± 0.08 mg/L, 0.62± 0.06 mg/L and 0.69± 0.10 mg/L for Gimbawa, Saminaka and Zaria reservoirs respectively (Table 2). These differences were however not significant between reservoirs, months and seasons (P > 0.05).

	Gimbawa			**Saminaka**			**Zaria**			**MPL**
	Min	**Max**	**Mean ± SE**	**Min**	**Max**	**Mean ± SE**	**Min**	**Max**	**Mean ± SE**	
Copper (mg/L)	ND	0.34	0.03 ± 0.03	ND	0.25	0.03 ± 0.02	ND	0.39	0.04 ± 0.03	2mg/L*
Zinc (mg/L)	ND	0.3	0.03 ± 0.03	ND	0.17	0.02± 0.01	ND	0.5	0.04 ± 0.04	3mg/L*
Manganese (mg/L)	ND	1.01	0.08 ±0.08	ND	0.58	0.09 ± 0.06	ND	0.72	0.06 ± 0.06	0.5mg/L*
Cadmium (mg/L)	0.06	0.22	0.14± 0.01	0.06	1.87	0.16 ± 0.02	0.06	0.25	0.11 ± 0.02	0.003mg/L*
Iron (mg/L)	ND	1.14	0.28 ± 0.1	ND	5.5	0.89 ± 0.43	ND	3.55	0.51 ± 0.28	0.3mg/L*
Nickel (mg/L)	0.17	1.06	0.64 ± 0.08	0.26	1	0.62 ± 0.06	0.17	1.17	0.69 ± 0.10	0.02mg/L*
Chromium (mg/L)	ND	0.96	0.43 ± 0.07	ND	0.67	0.36± 0.06	ND	1.1	0.34 ± 0.08	0.05mg/L*
Calcium (mg/L)	2.33	41.67	7.70 ± 3.10	1	20	6.4 ± 1.93	1	40	5.6 ± 3.14	200mg/L*
Magnesium (mg/L)	1.6	4.7	3.01 ± 0.24	0.9	8.3	3.19± 0.74	0.8	5.1	2.59 ± 0.31	0.02mg/L**
Potassium (mg/L)	2.6	8.5	4.80 ± 0.56	2.4	9.4	4.8 ± 0.55	2.8	6	4.2 ± 0.26	200mg/L*
Sodium (mg/L)	8.9	14.5	12.19 ± 0.53	6.4	27.3	11.49 ± 1.75	6.8	15.9	9.84 ± 0.74	200mg/L*

ND= not detectable, Min= minimum, Max= maximum, SE= Standard Error *WHO, 2006 ** Standard Organisation of Nigeria, 2007, MPL = maximum permissible limit

Table 2. Mean Values of Metal ions Observed in Gimbawa, Saminaka and Zaria reservoirs

The highest concentrations of 1.01, 0.58 and 0.5 mg/L of Manganese were observed in Gimbawa, Saminaka and Zaria reservoirs, the lowest concentrations of Manganese were below detectable limits in the three reservoirs. The mean ± SE concentration of Manganese was 0.08 ± 0.08mg/L,0.09±0.06 mg/L and 0.11±0.06 mg/L in Gimbawa, Saminaka and Zaria reservoirs respectively (Table 2).

The concentrations of Cadmium in the three reservoirs showed highest values of 0.22, 0.25 and 0.19 mg/L in Gimbawa, Saminaka and Zaria reservoirs respectively. The three reservoirs had lowest concentrations of 0.06 mg/L during the study period. The mean ± Standard Error for the reservoirs were 0.14± 0.01 mg/L, 0.16± 0.02 mg/L and 0.11± 0.02 mg/L for Gimbawa, Saminaka and Zaria reservoirs respectively (Table 2). These differences were however not significant between reservoirs, months and seasons ($P > 0.05$).

Magnesium concentration in the three reservoirs showed a highest concentration of 4.7mg/L, 8.3mg/L and 5.1mg/L and lowest of 1.6, 0.9 and 0.8 for Gimbawa, Saminaka and Zaria reservoirs respectively. The mean ± SE for the reservoirs were 7.70± 3.10 mg/L, 6.4± 1.93 mg/L and 5.6± 3.14 mg/L for Gimbawa, Saminaka and Zaria reservoirs respectively (Table 2). These differences were however not significant between reservoirs and months ($P > 0.05$) but significant between seasons ($P < 0.05$).

The highest Sodium concentrations observed were 14.5, 27.3 and 15.9 mg/L and lowest of 8.9, 6.4, and 6.8 mg/l in Gimbawa, Saminaka and Zaria reservoirs respectively. The mean ± SE for the reservoirs were 12.19± 0.53 mg/L, 11.49± 1.75 mg/L and 9.84± 0.74 mg/L for Gimbawa, Saminaka and Zaria reservoirs respectively (Table 2). These differences were however not significant between reservoirs and months ($P > 0.05$) but significant between seasons ($P < 0.05$), with significant interaction between reservoirs and seasons ($P < 0.01$).

Gimbawa, Saminaka and Zaria reservoirs had the highest concentration of Potassium of 8.5, 9.4 and 6 mg/L and lowest of 2.6, 2.4 and 2.8 mg/L respectively. The mean ± Standard Error for the reservoirs were 4.80± 0.56 mg/L, 4.80± 0.53 mg/L and 4.2± 0.26 mg/L for Gimbawa, Saminaka and Zaria reservoirs respectively (Table 2). These differences were however not significant between reservoirs, months and seasons ($P > 0.05$).

The three reservoirs had the highest Iron concentrations of 1.14 mg/L (Gimbawa), 5.4 mg/L (Saminaka) and 3.55mg/L (Zaria). The lowest concentrations of Iron were below detectable limits in the three reservoirs. The mean ± Standard Error for the reservoirs were 0.28± 0.1 mg/L, 0.89± 0.43 mg/L and 0.51± 0.28 mg/L for Gimbawa, Saminaka and Zaria reservoirs respectively (Table 2). These differences were however not significant between reservoirs, months and seasons ($P > 0.05$).

4.2 Cyanobacteria

Gimbawa reservoir had its highest number of cyanobacteria cells/L in the month of December (112) and lowest in the month June and August (0 cells/L). Saminaka reservoir had its highest number in the month of March (292 cells/L) and lowest in the months of June and January

(4cells/L). Zaria reservoir had its highest abundance in October (88cells/L) and lowest in the month of May (32 cells/L) (Table 3).

Number of taxa (8), number of individuals (308), Shannon Index (1.59) and Simpson index (0.76) was observed in Gimbawa reservoir during the dry season was higher than that observed in the wet season (4, 152, 1.11 and 0.62 respectively). Dominance was higher in the wet season (0.38) than dry season (0.24).

Reservoir	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Total
Gimbawa	16	0	28	0	68	12	60	112	12	40	40	44	432
Saminaka	24	12	40	32	60	40	48	52	4	68	292	220	892
Zaria	32	68	36	36	88	188	120	76	68	76	48	4	840

Table 3. Monthly Abundance (cells/L) of Cyanobacteria in Gimbawa, Saminaka and Zaria reservoirs

In Saminaka reservoir the trend was similar, number of individuals (464), Shannon Index (0.97) and Simpson index (0.56) observed during the dry season was higher than that observed in the wet season (236, 0.81 and 0.41 respectively), dominance was higher in the wet season (0.38) than dry season (0.24). The only exception was that the number of taxa observed in both seasons was equal (5).

Diversity Index	Gimbawa		Saminaka		Zaria	
	Wet	Dry	Wet	Dry	Wet	Dry
Taxa_S	4	8	5	5	4	6
Individuals	152	308	236	464	448	392
Dominance_D	0.38	0.24	0.59	0.44	0.53	0.31
Shannon_H	1.11	1.59	0.81	0.97	0.88	1.38
Simpson_1-D	0.62	0.76	0.41	0.56	0.47	0.69

Table 4. Seasonal Diversity Indices of Cyanobacteria in Gimbawa, Saminaka and Zaria reservoirs

In Zaria reservoir, the dry season a higher number of taxa (6), Shannon index (1.4) and Simpson index (0.69) were observed than the wet season (4, 0.88 and 0.47 respectively). While dominance (0.53) and number of individuals (448) observed in the wet season were higher than that observed in the dry season (0.31 and 392 respectively) (Table 4).

4.3 Relationship between physico-chemical characteristics and phytoplankton

In Gimbawa reservoir significant positive correlation was observed between Mg and *Sacconema* sp (r = 0.43) and *Trichodesmium* sp (r = 0.43) and between Fe and *Arthrospira* sp (0.43) and *Borzia* sp (0.43) (Table 5). pH and Electrical Conductivity showed significant positive correlation with *Arthrospira* sp (0.75 and 0.98 respectively); *Borzia* sp (0.75 and 0.98

respectively) and *Merismopedia* sp (0.51 and 0.64 respectively); BOD with *Merismopedia* sp (0.55) (Table 6).

In Saminaka reservoir, significant positive correlation was observed between Chromium with *Oscillatoria* sp (r = 0.40); Nickel with *Gleocystis* sp (r = 0.63), *Microcystis* sp (0.67) and *Trichodesmium* sp (0.45); Cadmium with *Gleocystis* sp (r = 0.82), *Microcystis* sp (0.88) and Iron with *Microcystis* sp (0.66). Significant negative correlation was observed between Potassium and *Spirulina* sp (-0.45); Sodium with *Oscillatoria* sp (-0.48) and *Sacconema* sp (-0.64); Chromium with *Merismopedia* sp (-0.49) (Table 5). *Microcystis* sp was observed to show significant positive correlation with DO (0.42), BOD (0.49), Alkalinity (0.64), NO_3-N (0.45). It showed significant negative correlation with Transparency (-0.40) and PO_4-P (-0.54). *Oscillatoria* sp showed significant positive correlation with Air Temperature (0.53), DO (0.50) and Alkalinity (0.50). *Spirulina* sp showed significant positive correlation with BOD (0.52) (Table 6).

	K	Na	Mg	Cr	Ni	Cd	Fe
Gimbawa							
Arthrospira sp	0.13	-0.12	0.04	-0.13	0.07	0.36	0.43*
Borzia sp	0.13	-0.12	0.04	-0.13	0.07	0.36	0.43*
Merismopedia sp	-0.09	0.11	-0.09	0.15	-0.06	0.1	0.24
Oscillatoria sp	-0.35	0.23	-0.15	0.18	-0.3	-0.22	0.04
Sacconema sp	0.01	-0.12	0.43*	0.1	-0.08	0.12	-0.11
Spirulina sp	0.34	-0.18	-0.06	-0.39	0.14	0.26	0.17
Spondylosium sp	-0.36	-0.3	-0.04	-0.3	0.29	0.25	-0.23
Trichodesmium sp	0.01	-0.12	0.43*	0.1	-0.08	0.12	-0.11
Saminaka							
Gleocystis sp	-0.19	0.02	0.08	0.24	0.63**	0.82**	0.76
Merismopedia sp	0.11	0.55*	0.33	-0.49*	0.07	0.04	0.12
Microcystis sp	-0.24	-0.17	0.10	0.13	0.67**	0.88**	0.66**
Nostoc sp	0.12	0.26	0.02	-0.18	0.05	-0.05	-0.02
Oscillatoria sp	-0.11	-0.48*	-0.31	0.40*	0.19	-0.22	0.29
Rivularia sp	-0.04	0.20	-0.18	-0.31	-0.27	-0.25	0.02
Saccconema sp	0.35	-0.64*	0.34	0.01	-0.32	-0.19	-0.21
Spirulina sp	-0.45*	-0.33	-0.08	-0.12	-0.31	-0.10	-0.26
Spondilosium sp	0.13	0.28	0.06	-0.01	0.28	0.30	-0.16
Trichodesmium sp	0.27	0.09	-0.04	-0.01	0.45*	0.08	-0.01
Zaria							
Merismopedia **sp**	-0.38	-0.02	-0.14	-0.20	-0.18	-0.31	-0.16
Nostoc **sp**	0.12	0.26	0.02	-0.18	0.05	-0.05	-0.02
Oscillatoria **sp**	-0.11	-0.48**	-0.31	0.40*	0.19	-0.22	0.29
Sacconema **sp**	-0.49*	0.03	-0.15	-0.18	0.05	-0.01	-0.21
Spirulina **sp**	-0.45*	-0.33	-0.08	-0.12	-0.31	-0.10	-0.26
Spondilosium **sp**	0.13	0.28	0.06	-0.01	0.28	0.30	-0.16
Trichodesmium **sp**	0.27	0.09	-0.04	-0.01	0.45	0.08	-0.01

*Significant $P < 0.05$, ** Significant $P < 0.05$

Table 5. Correlation Coefficient (r) between Cyanobacteria and Metal Ion Concentration in Gimbawa, Saminaka and Zaria reservoirs

In Zaria reservoir, Significant negative correlation was observed between Potassium and *Sacconema* sp (r = -0.49), *Spirulina* sp (r = -0.45); Sodium with *Oscillatoria* sp (r = -0.48). Chromium showed significant positive correlation with *Oscillatoria* sp (0.40) (Table 5).

Species	Air Temp	Water Temp	Transp	pH	EC	DO	BOD	Alkalinity	Hardness	$N0_3$-N	$P0_4$-P
Gimbawa											
Arthrospira sp	0.08	0.32	-0.26	0.75*	0.98**	0.25	0.25	0.27	-0.11	-0.04	0.22
Borzia sp	0.08	0.32	-0.26	0.75*	0.98**	0.25	0.25	0.27	-0.11	-0.04	0.22
Merismopedia sp	0.31	0.33	0.02	0.51*	0.64**	0.32	0.55**	0.04	0.00	-0.07	0.23
Saminaka											
Microcystis sp	0.13	0.08	-0.40*	0.37	0.34	0.42*	0.49*	0.64**	0.03	0.45*	-0.54**
Oscillatoria sp	0.53**	0.18	0.35	0.29	0.12	0.50**	0.27	0.50**	-0.15	0.14	0.15
Spirulina sp	0.14	0.39	-0.06	0.22	0.05	0.08	0.52**	0.28	-0.12	0.26	-0.01
Zaria											
Nostoc sp	-0.50**	-0.80**	0.62**	0.36	-0.15	0.11	0.02	0.14	0.00	-0.01	-0.39
Spirulina sp	0.14	0.24	-0.22	-0.29	-0.20	0.00	0.01	-0.15	-0.28	-0.09	0.48*
Spondilosium sp	0.76**	0.23	-0.02	0.20	-0.06	0.59**	0.44*	0.45*	-0.22	0.02	0.19

*Significant $P < 0.05$, **Significant $P < 0.05$

Table 6. Correlation Coefficient between Cyanobacteria and Physico-chemical Parameters in Gimbawa, Saminaka and Zaria reservoirs

5. Discussion

The statistically significant monthly variation of mean Air Temperature in the three reservoirs could be attributed to the low temperatures experienced between the months of November and February as a result of the harmattan wind (Ezra and Nwankwo, 2001). The significantly higher Transparency of the water in Gimbawa reservoir may be attributed to the low human pressure in its catchment as it is location in the outskirts of a major human settlement. Thus, receiving low amount of silt and nutrients that stimulate algal and cyanobacterial growth. Silt and plankton abundance have been implicated in Transparency fluctuations (Davies *et al*, 2009). The circum-neutral mean pH of water in the reservoirs may be attributed to the relatively high alkalinity values of the reservoirs, which is effective as a buffer to fluctuations of pH that might be caused by introduction of waste water, photosynthesis and other metabolic processes. The wide fluctuations of EC (SE in the range of 38.59 to 41.95) an significantly monthly variations in the three reservoirs may be attributed to concentration of Electrical Conducting ions due to evaporation during the extensive (six) months of dry season. Similar results were obtained by Chia and Bako (2008). DO concentration was found within the limit of 5-9 mg/l, drinking water (UNESCO/WHO/UNEP, 1996). The mean BOD values of Gimbawa and Saminaka were slightly above the 2 mg/l. BOD above 2 mg/l is associated with waste water contamination (UNESCO/WHO/UNEP, 1996). The mean hardness values (< 1.5 mg/l) may be due to the uptake of the ions (calcium and magnesium) responsible for harness of water by aquatic organisms. Calcium and Magnesium are essential for aquatic organisms such as algae, cyanobacteria, aquatic macrophytes as well as other reptiles. The Mean NO_3-N (1.2 and 1.3 mg/l in Gimbawa and Zaria reservoirs) and PO_4-P (0.29 in Gimbawa reservoir and 0.39 in Saminaka and Zaria reservoirs) were found to be above expected concentration range of natural unpolluted waters of 0.1mg/l and 0.001mg/l respectively, and are capable of stimulating cyanobacterial bloom (UNESCO/WHO/UNEP, 1996).

Metal ions such as Manganese, Iron, Cadmium, Nickel, Chromium, Magnesium showed concentrations higher than the maximum permissible limit for WHO (2006) and SON (2007), other metals like Copper, Zinc, Sodium and Potassium were found to be below the maximum permissible limit. The implication of high concentrations of metal ions in drinking water include: Manganese causes neurological disorder and at concentrations exceeding 0.1mg/L it causes undesirable taste in beverages, stains laundry and may lead to the accumulation of deposit in water distribution system. Iron at levels above 0.3mg/L stains laundry and plumbing fixtures (WHO, 2006). Cadmium is toxic to the kidney, Chromium is carcinogenic, and Magnesium affects consumer acceptability of drinking water (SON, 2007). Zinc imparts an undesirable astringent taste to water at threshold concentration of 4mg/L, water containing Zinc at excess of 3-5mg/L may appear apalacent and develop greasy film on boiling (WHO, 2006). With the exception of Magnesium, all the others are heavy metals capable of accumulating in increasing concentration as they move up the food chain (Chindah *et al*, 2004).

The dynamics of the concentration of these metals may be attributed to inflow of waste water from residential areas (as they are components of many household products such as pesticides, fungicides, paints, batteries and plumbing facilities), remobilization from sediment due to fluctuations of pH, inflow of agro-chemicals such as fertilizers, pesticides and herbicides from farms in the catchment of the reservoirs, chemicals from washing of automobiles. Changes in pH affects the solubility of metal ions, lowering of pH may remobilize insoluble metal complexes adsorbed on clay and silica in the sediments into the water column, for example at pH 6.7, Zinc is available to form complexes with organic matter while at pH > 7 Zinc begin to hydrolyze and form stable $Zn(OH)_2$ at pH 8. It is important to note that there is a difference between the presence of a metal and its bioavailability. A metal may be present in a form that is not available for utilization by algae and other organisms (Kalis, 2006).

The variation in abundance of Cyanobacteria (Saminaka > Zaria > Gimbawa) in the reservoirs during the study period may be attributed to the variation in N: P ratio (Gimbawa, 0.41; Saminaka, 0.23 and Zaria, 0.33) of the water bodies. Lower N: P ratio promotes cyanobacteria abundance (Tisser *et al.*, 2008). They take advantage of their ability to fix nitrogen into the aquatic environment, thus enabling them to out-compete other divisions (Chorus and Batram, 1999). High phosphate concentration may result from detergents from sewage, washing of cars, clothes and from fertilization of farms in the catchment of the reservoirs (Reynolds, 1998). High abundance of Cyanobacteria in drinking water may be a serious problem in drinking water as they produce toxins which are harmful to fish, livestock, other aquatic organisms and ultimately man (WHO, 2006). The presence of a bloom of species *Microcystis*, a toxin producing genus in the Saminaka reservoir is worrisome.

The differences in number of taxa and number of individuals between seasons may be due differences in temperatures and pH as different species obtain nutrition at different pH and temperatures. Wilm and Dorris (1966) have suggested a relationship between species diversity and pollution status of aquatic system and classified as follows; > 1 = Clean water, 1-3 = moderately-polluted < 1 = Heavily- polluted. Based on this classification, Gimbawa reservoir was moderately polluted in both seasons, Saminaka reservoir heavily polluted in both seasons while the Zaria reservoir was heavily polluted in the wet season and moderately polluted in the dry season. A similar classification was also used by Shehata *et. al.* (2009). Simpson index gives the evenness of species distribution; the higher evenness in species distribution in the dry season may be an indication that the water quality was better to support the growth of most of the species observed.

Significant positive correlation between cyanobacteria and metal ions may be an indication of the possibility of using as indicators of the levels of these metals in a water body. These metals are either essential (Fe, Cu, Zn, Na, Ca, Mn, Co and K) or beneficial (Ni and As) in phytoplankton physiological processes (Paerles-Vela, *et al*., 2006). Significant negative correlation between metal ions and cyanobacteria may be an indication of toxicity of these metals at high concentrations level exceeding the requirement for nutrition or increased utilization of metals in periods of high abundance. Some of the metals that show significant negative correlation with cyanobacteria abundance are either essential (Fe, Cu, Zn, Na, Ca, Mn, Co and K) or beneficial (Ni and As) in cyanobacteria physiological processes (Daffus, 2002). Significant negative correlation between metal ions and cyanobacteria may be an indication of toxicity of these metals at high concentrations level exceeding the requirement for nutrition or increased utilization of metals in periods of high abundance ((Paerles-Vela, *et al*., 2006).

Significant positive correlation between cyanobacteria with pH and alkalinity may be due to the fact that some essential elements are bioavailable at certain required pH, on the other hand, the bioavailability of certain elements at toxic concentrations as affected by pH may cause a significant negative correlation between pH and alkalinity with cyanobacterial abundance. A significant positive correlation between nutrient (N and P) load and cyanobacteria abundance may be due to the fact that increased nutrient concentrations leads to a resultant increase in cyanobacteria abundance and a significant negative correlation may due to the reason that increased cyanobacteria abundance may lead to increased utilization of such nutrients by cyanobacteria (Rabalais, 2002). The significant relationship(s) between cyanobacteria abundance and DO, BOD, EC, Hardness, Temperature and Transparency is an indication of the inter-dependance between these important water quality characteristics and the Biota (Shehata *et al*., 2009).

6. Conclusion

The introduction of waste water into these reservoirs greatly impairs the water quality of these reservoirs. The consequence is seen as the elevated concentration of heavy metals such as Cadmium, Iron, Nickel and Chromium above WHO permissible limit in drinking water. Waste water is also implicated in the increased nutrient (N and P) levels. These nutrients were found to be below concentrations to cause any harm directly to consumers but indirectly by their ability to stimulate cyanobacterial growth. Shannon-Weiner diversity index showed that the water quality of the three reservoirs follows this order Gimbawa > Zaria > Saminaka.

7. References

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14

Water Quality of Streams Receiving Municipal Waste Water in Port Harcourt, Niger Delta, Nigeria

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1. Introduction

The Niger Delta environment was relatively a pristine area some 100 years ago and consists of several ecological zones mainly lowland forest, freshwater swamp forest, prominent in the northern limit while the mangrove and barrier island zones dominate the southern stretch (RPI, 1985, NDES, 2000 and NDDC, 2004). Settlements were of small population and largely in pockets around these ecological zones. The people are agrarian and indulge mostly in farming, fishing and exploitation of timber and non timber forest products. With the relative small nature of the populations in the settlements their wastes generated and discharged into the environment had little or no significant impact on the environment (Onuoha, *et. al.*, 1991).

With the absence of pipe borne water they depended on the stream system for the potable water use, recreation, washing, bathing and fishing (Amadi *et. al.*, 1997).

The advent of civilization has attracted human population to the major urban centres for white collar jobs and more also the crude oil found in commercial quantity in the region has accelerated the pace of development in terms of human population, urban growth, industrial activities, infrastructural development, intensive farming and other economic activities (NDDC,2004, Petrarova *et. al.*, 2009, Onderka *et. al.*, 2010).

The growth of human population and rapid industrialization led to increasing use of urban waters as sewers, compromising their other uses. The discharge of industrial effluents has led inevitably, to alterations in the quality and ecology of receiving water bodies (Sheikh, and Irshad. 1980 and Wahid *et. al.*, 1999). This results into new challenges to water resource managers and aquatic ecologists. Several attempts have been made to regulate/control the quality of effluents that are discharged from waste generating industries into the water systems with little effort on urban discharges. Today, most urban areas of the developing world remain inadequately served by sewage treatment infrastructure (NDDC,2004). Untreated wastes pose serious threats to associated environment including human health risks. Commonly cited effects of industrial effluents on the receiving waters are high turbidity, reduced transparency, increased suspended solids and oxygen depletion (Rafiu *et. al.*, 2007). The area study covers over 94.72 km^2 with a population of about 1.9 million.

The tremendous spatial spread of the Port Harcourt city has resulted in land take for various purposes, encroached, and converted to build up area with concrete buildings by both the government and private agencies without providing open spaces and corridors. Consequently, the natural water bodies (stream) draining the forest seawards are left bare including the stream banks especially in Port Harcourt area where this study was undertaken. As a result the water bodies now lack ecotonal characteristics required to undertake self-purification through biological processes (Lakatos et al 1997, Soler et al. 1991, Chindah *et. al.*, 2007).

It is known that these water bodies have that drain Port Harcourt Municipality played a crucial role in growth and sustaining the development of human communities; however, it is paradoxical that they have undergone degradation in modern times due to various anthropogenic activities (Chindah *et. al.*, 2009). With the attendant increase in population, industrial and commercial activities, untreated municipal, industrial solid wastes and effluents discharged have led to the total degradation of the water quality in many of the stream systems(Ogan, 1988, Ogamba *et. al.,* and Omunakwe *et. al.*, 2009). The consequences include the problem of water pollution rendering water no longer fit for drinking, recreation, as well as for aquatic life. As a result, thousands of children die everyday from diarrhoea and other water, sanitation and hygiene related diseases and many suffer and are weakened by illness (Pandey, 2006). Streams and rivers are vital and vulnerable freshwater systems that are critical for the sustenance of all life. However, the declining quality of the water in these systems threatens their sustainability and is therefore a cause for concern despite their importance in providing various water resources for domestic, industrial, and agricultural purposes (Musaddiq, 2002, Qureshi and Dutka, 1979).

The objective of this research is to find out the pollution level of different streams located in these catchments. The pollution level was determined by examining different physical and chemical parameters of waste water such as Temperature, pH, alkalinity, hardness, dissolved oxygen, BOD, Ammonia, Nitrate, Sulphate and phosphate, and microbiological properties.

2. Materials and methods

2.1 The study area:

Geographically, Port Harcourt is situated at the eastern flank of the New Calabar River, the study streams are located within the lowland and freshwater swamp zones that accommodate the northern limits of Port Harcourt City and lie between latitude 4^{o} 43′E – 4^{o} 50′ E and longitude 6^{o} 57′N – 7^{o} 05′N. The catchments cover an estimated area of 94.72km^2 made of flat ground with lithosphere and hydrosphere are interrelated and consequently involved closely related problems including non point source pollutants.

The five streams that drain this catchment and finally empty into Bonny estuary include:-

- Ntawogba Stream
- Miniweja Stream
- Miniokoro Stream
- Minichida Stream and
- Agbonchia Stream.

Ntawogba Stream

Ntawogba stream lies on the extreme west of the municipality and drains the marshy swamp forest up stream of (Rumueme and Rumuepirikom) empties into Amadi creek.

Large ares of the catchment are developed with concrete structures and the lower reach of the stream is concreted.

Miniweja Stream

This stream system drains the Freshwater (Rumuigbo/Rumuola) forest and through various communities and empties into Diobu Creek of Bonny estuary. The greater stretch of the stream channel is degraded while the middle and lower reaches have been more developed with concrete structures.

Miniokoro Stream

Miniokoro stream drains the freshwater swamp forest into Woji creek from where it eventually empties into the Bonny estuary. The entire stream stretch is degraded, built up area with concrete structures and paved roads.

Minichida Stream

Minichida drains the freshwater swamp forest, meanders through communities and empties into Elelenwo creek in Bonny estuary. All the reaches of the stream are degraded by human activities with concrete structures along the water course.

Agbonchia Stream

Agboncha which lies in the east flank of Port Harcourt drains the freshwater swamp forest and empties through Obufe /Elelenwo creek into the Bonny estuary. Development and degradation is fairly low compared with other stream systems but the water body also read recieives effluent of a petrochemical plant.

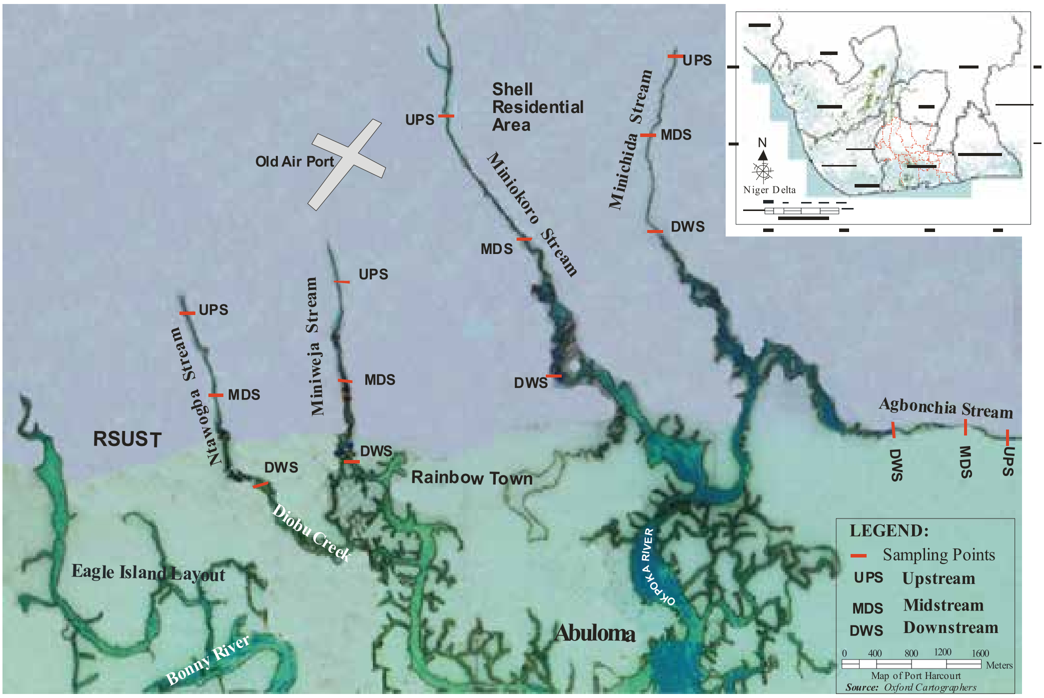

Fig. 1. Map of Niger Delta, Port Harcourt environment showing the 5 study streams

The climatic condition is humid typical of the semi hot equatorial type (Gobo, 1988 and Gobo *et. al.*, 2008). The area experiences heavy rainfall from April to October. Sporadic rainfalls are however experienced during the dry season months of November to March. The mean annual rainfall is estimated to be about 2,405 mm. The prime cause of critical unsanitary conditions of the water bodies is due to the lack of facilities for collection and disposal of waste effectively such that municipal untreated effluent wastewater are discharged into natural surface water drains and sometimes on land and finally through storm water to the stream systems

2.2 Sampling strategy

Samples were collected monthly for 12 months to cover the two main seasons from 6 designated locations from each of the 5 streams (Agboncha stream, Minichida stream, Miniokoro stream, Miniweja stream and Nta-wogba stream). Each of the stream systems, were strategically divided into three segments (upper limit, middle reach and down stream) along the water course considering flow, stretch and human activities. For each segment, two stations were located at an interval of 0.5km. The parameters sampled include temperature, pH, alkalinity, hardness, conductivity, dissolved oxygen, BOD_5, sulphate, ammonia, nitrate, phosphate and microbial properties.

2.3 Physicochemical parameters

In the field, samples for each station were collected with clean 2ml plastic containers at sub-surface level and stored in an ice chest (-4oC). The samples was immediately transferred to the Institute of Pollution Studies (IPS) laboratory for analysis. In the laboratory, analysis was done using procedures as outlined in Standard Methods for the Examination of water and wastewater (1 and 10). Temperature was measured in-situ using a mercury bulb thermometer. pH was measured with a pH meter (Hanna instrument model HI8314). The conductivity was measured using the Horiba water checker model U-10 and Carbon dioxide was measured by the tirimetric method as described in APHA (1998). Dissolved oxygen (DO), and biochemical oxygen demand (BOD_5) were determined using Winkler's method as described in APHA (1998). Other parameters such as ammonia-nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), sulphate (SO_4^{-2}), and phosphate (PO_4^{-3}) concentrations were determined spectrophotometrically (Spectronic Spectrophotometer 21D), following the procedures as described in APHA (1998). The media used for the bacteriological analysis of water include plate count agar (PCA), nutrient agar (NA), lactose broth (LB), and Eosin Methylene blue agar (EMB). All the media used were weighed-out and prepared according to the manufacture's specification, with respect to the given instructions and directions. A serial dilution method was used for total viable count and the presumptive test for coliforms (APHA,1998).

3. Results

3.1 Minichida

Water temperature was generally high, as expected in the dry season (26.75 ± 1.1 - 27.25 ± 1.27^{o}C) value slightly higher than that of wet season (26.79 ± 0.99 - 26.93 ± 1.32^{o}C). The differences in temperature amongst stations were significant (R^2 = 1) but not significant during the wet season (R^2 = 0.25) Table 1.

		Nta-Wogba			Miniweja			Miniokoro			MiniChida			Agboncha		
Parameter	Season	Upstream	Middle Reach	Down Stream	Upstream	Middle Reach	Down Stream	Upstream	Middle Reach	Down Stream	Upstream	Middle Reach	Down Stream	Upstream	Middle Reach	Down Stream
T °C	DRY	28.00	28.50	31.00	28.13	30.17	30.58	26.84	28.17	30.33	26.75	27.00	27.25	26.96	26.83	27.17
	SD	1.00	1.05	1.91	0.93	1.60	1.49	1.04	1.94	1.12	1.10	1.26	1.27	1.38	0.41	0.73
	WET	28.07	27.50	28.64	26.72	28.29	28.32	26.22	27.29	29.25	26.86	26.79	26.93	26.43	26.43	26.47
	SD	2.83	1.44	2.63	1.13	2.06	2.49	1.42	1.60	1.40	1.08	0.99	1.32	1.13	1.13	1.12
pH	DRY	6.17	6.19	6.29	6.24	6.55	6.58	6.10	5.90	6.57	6.46	5.97	6.07	6.85	7.00	6.95
	SD	0.03	0.04	0.05	0.35	0.30	0.39	0.38	0.54	0.41	0.30	0.38	0.38	0.45	0.40	0.45
	WET	6.46	6.55	6.57	6.25	6.33	6.37	6.18	6.00	6.35	6.25	6.30	6.19	7.35	7.50	7.30
	SD	0.16	0.13	0.18	0.27	0.16	0.34	0.33	0.41	0.45	0.53	0.46	0.19	0.05	0.10	0.00
CO_2	DRY	24.42	11.79	12.66	35.10	26.39	24.33	23.90	31.75	18.57	43.55	33.98	35.23	19.73	31.21	29.24
(mg/l)	SD	16.48	4.49	8.90	9.59	4.63	9.02	9.87	12.28	5.50	14.54	8.91	9.57	6.34	10.30	3.18
	WET	36.96	38.10	25.83	40.88	37.67	36.74	38.67	39.67	25.23	32.89	37.96	32.61	22.48	31.96	32.50
	SD	16.20	19.52	11.88	13.37	13.62	17.07	26.63	26.97	6.23	9.51	12.07	10.86	4.65	11.34	9.05
ALKALINITY	DRY	28.10	155.33	60.50	11.84	28.00	32.50	7.17	11.00	31.84	7.17	7.67	7.00	4.50	6.33	7.00
(mg/l)	SD	21.62	144.35	18.06	2.86	13.86	23.65	1.87	2.45	8.31	2.89	1.51	2.43	1.45	1.97	3.05
	WET	21.72	46.29	51.50	12.07	20.57	24.72	7.00	7.43	23.86	7.57	7.71	11.14	4.22	5.86	6.57
	SD	10.24	22.22	19.48	3.22	10.05	10.88	2.56	3.41	10.31	2.33	2.69	4.26	2.10	2.34	2.46
CONDUCTIVITY	DRY	250.30	1215.00	25565.00	2263.85	13165.00	17190.85	33.34	221.00	1831.67	29.59	26.00	29.00	22.50	39.50	409.25
(µS/cm)	SD	271.46	1029.56	13961.91	2433.75	14532.40	16075.35	7.34	211.34	1223.84	5.77	5.55	6.25	8.48	13.90	415.15
	WET	99.14	224.29	12895.72	543.00	5994.30	7888.60	35.72	89.86	1053.57	33.86	28.86	42.29	27.67	37.29	459.00
	SD	38.13	87.53	10074.36	1196.95	11684.30	9742.30	16.22	132.68	1205.89	7.13	8.93	19.96	30.88	9.53	754.54
HARDNESS	DRY	38.50	184.96	2297.60	333.12	1076.80	1438.72	10.88	32.96	161.20	8.32	6.40	7.52	5.12	5.76	60.80
(mg/l)	SD	25.09	165.11	1128.16	335.97	1150.57	1367.80	9.88	20.73	80.45	5.02	3.36	3.44	2.87	2.97	76.12
	WET	27.02	63.36	1392.00	183.41	751.54	1380.35	19.06	20.30	137.62	12.21	9.34	16.46	4.93	5.21	107.66
	SD	11.52	24.51	848.51	287.88	857.59	1575.43	18.40	17.83	86.91	6.99	5.23	12.43	4.50	3.27	131.78
DO	DRY	1.45	0.44	5.80	3.24	5.97	6.91	2.10	5.16	5.36	1.48	1.10	1.18	2.88	5.04	5.46
(mg/l)	SD	1.59	0.71	4.45	1.01	2.25	3.06	1.02	0.93	1.89	0.71	0.71	0.62	0.94	0.82	1.21
	WET	6.03	4.41	6.41	3.64	6.24	6.44	2.56	4.99	5.12	4.10	3.05	2.19	2.97	4.81	4.90
	SD	7.67	2.62	3.04	1.20	2.11	2.93	0.88	0.94	1.55	2.56	2.62	1.52	0.85	0.74	0.64
BOD	DRY	26.45	34.82	55.25	18.72	23.82	25.56	23.28	29.16	33.85	11.78	18.66	27.76	9.62	14.39	17.32
(mg/l)	SD	9.67	9.43	7.44	5.74	4.84	6.58	3.59	4.17	5.85	6.55	3.41	7.47	0.95	0.97	0.92
	WET	13.45	26.23	37.86	11.65	13.07	14.62	12.92	19.16	22.66	9.52	12.64	16.49	5.75	11.39	19.83
	SD	3.50	8.66	8.54	5.83	5.53	6.67	4.67	2.05	5.63	2.74	2.57	3.13	3.77	2.90	5.90
NH_4-N	DRY	0.79	3.39	1.35	0.45	0.19	0.33	0.42	0.31	0.18	0.39	0.59	0.26	0.25	0.20	0.23
(mg/l)	SD	0.89	3.73	1.78	0.42	0.18	0.33	0.50	0.31	0.14	0.40	0.91	0.34	0.22	0.19	0.24
	WET	0.68	1.34	0.82	0.31	0.29	0.38	0.40	0.38	0.38	0.33	0.31	0.38	0.31	0.26	0.27
	SD	0.73	1.49	1.11	0.27	0.21	0.42	0.45	0.30	0.37	0.29	0.30	0.49	0.23	0.20	0.20
NO_3-N	DRY	0.67	0.43	0.53	0.81	0.71	0.68	0.55	0.91	0.66	0.56	0.46	0.46	0.53	0.60	0.58
(mg/l)	SD	0.41	0.20	0.28	0.31	0.22	0.18	0.24	0.39	0.28	0.17	0.20	0.17	0.28	0.37	0.25
	WET	0.52	0.55	0.71	0.91	0.61	0.69	0.43	0.35	0.49	0.41	0.35	0.38	0.33	0.45	0.35
	SD	0.14	0.21	0.21	1.33	0.27	0.24	0.17	0.16	0.22	0.21	0.25	0.20	0.19	0.51	0.22
SO_4^{2-}	DRY	15.50	13.38	275.32	61.81	414.50	603.06	0.91	1.12	45.53	0.76	0.75	0.75	1.69	5.50	21.90
(mg/l)	SD	13.15	6.44	194.26	70.84	359.93	486.01	0.20	0.31	29.30	0.38	0.37	0.37	1.58	2.73	24.45
	WET	9.92	6.29	227.50	18.64	165.04	199.91	0.92	1.03	34.25	0.95	0.90	0.85	1.36	57.51	7.43
	SD	8.74	3.94	124.57	42.17	288.78	272.36	0.19	0.10	21.78	0.09	0.00	0.20	0.76	38.72	7.29
PO_4- P	DRY	0.18	0.33	0.14	0.13	0.14	0.15	0.20	0.12	0.14	0.11	0.13	0.11	10.25	14.70	8.80
(mg/l)	SD	0.16	0.35	0.17	0.12	0.13	0.15	0.26	0.09	0.07	0.05	0.06	0.08	8.90	10.00	1.65
	WET	0.32	0.47	0.32	0.15	0.19	0.25	0.38	0.18	0.10	0.10	0.09	0.15	3.90	10.10	60.25
	SD	0.17	0.37	0.25	0.08	0.19	0.24	0.29	0.11	0.06	0.06	0.03	0.08	2.40	2.30	29.35
	SD	8.74	3.94	124.57	42.17	288.78	272.36	0.19	0.10	21.78	0.09	0.00	0.20	0.76	38.72	7.29
Total Coliform	DRY	677.00	578.00	986.00	342.00	459.00	533.00	235.12	298.82	466.81	189.67	311.56	289.34	78.69	84.55	210.63
(cfu/100ml)	SD	102.64	87.12	134.00	45.34	101.23	76.80	45.23	12.23	56.41	34.22	57.84	48.93	34.12	41.64	98.57
	WET	452.00	582.00	811.00	621.86	782.15	698.45	302.33	523.45	588.77	342.28	427.28	462.93	85.43	107.36	299.51
	SD	65.00	79.00	122.00	76.33	95.83	54.81	52.18	105.22	96.42	24.78	58.91	95.32	23.78	45.41	68.42
Faecal Coliform	DRY	192.66	225.66	328.67	114.00	153.00	177.54	78.37	99.61	155.60	63.22	103.85	96.45	26.23	28.18	70.21
(cfu/100ml)	SD	22.80	19.36	29.78	10.07	22.49	17.06	10.05	2.71	12.56	7.64	12.83	10.87	7.58	9.25	21.90
	WET	151.67	272.14	195.30	208.63	296.39	262.46	201.45	175.65	197.56	114.85	143.38	155.34	28.66	36.02	100.56
	SD	19.11	23.23	35.88	22.45	28.18	16.12	15.34	30.94	28.35	7.25	17.35	28.01	6.99	13.35	20.12

Table 1. The mean and standard deviation of the physicochemical and microbial variables for the 3 main ecological limits of the 5 streams for wet and dry seasons

pH was acidic for both seasons with values slightly more acidic in the dry season (5.97 ± 0.38- 6.46 ± 0.30) than in the wet (6.19 ± 0.19 - 6.30 ± 0.46). Concentrations of alkalinity increased from the upstream to the down stream stations for wet and dry seasons, Seasonal trend demonstrated higher concentrations in the wet season (7.57 + 2.33 - 11.14 ± 4.25mg/l) than during the dry (7.17 ± 2.89 - 8.10 ± 2.43mg/l) season. Spatial differences between the stations in dry season (R^2 = 0.06) were not significant but significant differences were observed during the wet season (R^2 = 0.78) amongst the stations. Carbon dioxide concentrations were relatively higher in the dry season (33.98 ± 8.91 - 43.55 ± 14.54mg/l) than in the wet season (32.61 ± 10.86 - 37.96 ± 12.07mg/l) Table 1.

Conductivity was relatively higher in the wet season (28.86 ± 8.93 - 42.29 ± 8.93µS/cm) than in the dry season (26.0 ± 5.55 - 29.59 ± 5.77 µS/cm) with no clear spatial trend along the water course. Alkaline value was higher in the wet season (7.57 ± 2.33 – 11.14 ± 4.16mg/l) than during the dry season (7.0 ± 2.43 - 7.67 ± 1.51mg/l) with concentrations increasing down stream in the wet but in the dry season no defined sequence was observed and the spatial distributions were not significant in the dry season (R^2 = 0.06) but significance was observed in the wet season (R^2 = 0.78).

Hardness values were higher in the wet season (9.34 + 5.23 - 16.46 + 12.43mg/l) than during the dry season (6.4 ± 3.36 - 8.32 ± 5.02mg/l) and there were no clear spatial patterns between wet and dry seasons Affinity between the stations for wet and dry seasons were not significant but the dry season (R^2 = 0.35) values indicated closer affinity between stations than in the wet season (R^2 = 0.17). Dissolved oxygen concentrations were low in the dry season (1.1 ± 0.71 - 1.48 ± 0.71mg/l) than during the wet season (2.19 ± 1.52 - 4.1 ± 2.58 mg/) with the upstream having the highest dissolved oxygen concentrations than the other limits for both seasons.

BOD_5 on the other hand recorded higher concentrations in the dry season (11.78 ± 6.55 - 27.76 ± 7.47mg/l) than in the wet season (9.52 ± 2.74 - 16.49 ± 3.13mg/l).in both seasons, concentrations increased down stream.

Sulphate concentrations tended to decrease down stream for both seasons with wet season (0.85 ± 0.20 - 0.95 ± 0.09mg/l) concentrations being higher than dry season values (0.75 ±0.37 - 0.76 ± 0.38mg/l). However, the distribution of concentrations between the stations indicated closer affinity in the wet season (R^2 = 1) than during the dry season (R^2 = 0.75)

Ammonia nitrogen concentrations were relatively higher in the dry season (0.28 ± 0.34 - 0.59 ± 0.91mg/l) than in the wet season (0.31 ± 0.30 - 0.38 ± 0.49mg/l) and the spatial distribution demonstrated that the middle reach had higher concentrations followed by the upstream and the down stream in that respective order (Table 1).

Nitrate nitrogen concentrations declined downstream with concentrations being relatively higher in the dry season (0.45 ± 0.17 - 0.56 ± 0.17mg/l) than during the wet season (0.35±0.25 - 0.41 ± 0.21mg/l). Also differences between the stations in dry season (R^2 = 0.79) were significant but significance was not observed in the wet season (R^2 = 0.29). Phosphate concentrations were low for both seasons dry season concentrations (0.11± 0.05 - 0.13 ± 0.06mg/l) being slightly higher than wet season concentrations (0.01 ± 0.03 - 0.15 ± 0.08mg/l) Table 1.

The total coliform concentrations were high for both seasons and concentrations consistently increased down stream. Dry season (189.67 ± 34.22 – 289.34 ±48.93 cfu/100ml) concentrations were higher than values observed in the wet season (342.28 ±24.78 – 462.93 ±95.32cfu/100ml). Spatial distribution amongst the three zones indicated significance but with closer affinity between the zones in dry season (R^2 = 0.95) than in the wet season

(R^2 = 0.59). Correspondingly, the faecal coliform concentrations followed similar seasonal and spatial pattern as observed but concentrations were lower by a magnitude of about 4 times with concentrations for dry season (63.22 ± 7.64 – 103.85 ± 12.83cfu/100ml) being higher than of the wet season (114.85 ± 7.25 – 155.34 ±28.01cfu/100ml) Table 1.

3.2 Agbonchia

Temperature values were high with wet season (26.43 ± 1.13 - 26.47 ± 1.12°C) values not remarkably different from the dry season (26.83 ± 1.38 - 27.17 ± 0.73°C) but spatially distribution amongst the study stations indicated significant difference in wet season (R^2 = 0.75) while dry season temperature distributions were not significant (R^2 = 0.39) Table 1. In dry season water pH ranged from slightly acidic to neutral while wet season pH was for all the stations, above neutral value. Spatial distributions amongst the stations were significant in dry season (R^2 = 0.99) indicating differences in distribution while wet season values were not significant (R^2 = 0.25).

Carbon dioxide concentrations were considerably higher in the dry season than in the wet season with values almost increasing down stream for both seasons and differences between the stations were significant for wet (R^2 = 0.79) and dry season (R^2 = 0.60). Dy season concentrations demonstrated closer affinity than that of wet season (Table 1).

Alkalinity values for both seasons increased down stream and were relatively higher in the dry season (4.50 ± 1.45 - 7.0 ± 3.05 mg/l) than during the wet season (4.22 ± 2.1 - 6.57 ± 2.46mg/l). Spatial differences between stations were positively significant for wet (R^2 = 0.95) and dry season (R^2 = 0.93). Similarly water hardness increased down stream for both seasons and concentrations were higher in the wet season (4.93 ± 4.50 - 107.66 ± 131.78mg/l) than during the dry season (5.12 ± 2.87 - 60.80 ± 76.12mg/l). The distribution between the stations were significant for wet (R^2 = 0.75) and dry (R^2 = 0.76) seasons.

Highest conductivity concentrations were observed at the down stream stations which are about 40 - 50 times higher than values observed for the other stations for both seasons. Concentrations for wet season were relatively higher in the wet (27.67 ± 30.88 - 459 ± 755.54µS/cm) than in the dry season (22.50 ± 8.48 - 409 ± 459.15 µS/cm). Spatial differences between the stations was significant in wet season (R^2 = 0.56) but not significant in the dry season (R^2 = 0.20) Table 1.

Dissolved oxygen concentrations were low and generally increased down stream for both seasons with dry season concentrations generally higher (2.88 ± 0.94 - 5.46± 1.21mg/l) than in the wet (2.97 ± 0.85 - 4.90 ± 0.64mg/l). Spatial differences between the stations for wet (R^2 = 0.78) and dry seasons were significant (R^2 = 0.87) Table 1.

BOD_5 values were considerably high for both wet (5.75 ± 3.77 - 16.83 ± 5.90mg/l) and dry (9.62 ± 0.95 - 17.32 ± 0.90mg/l) seasons. The values consistently season increased down stream in dry season, similarly wet season concentrations at the down stream stations the recorded highest values. However spatial variations between the stations indicated marked differences between the stations for dry (R^2 = 0.92) and wet season (R^2 = 0.69) Table 1.

Ammonia concentrations were low for both seasons with wet season (0.26 ± 0.20 - 0.31 ± 0.23mg/l) concentrations being higher than in of the dry season (0.20 ± 0.19 - 0.25 ± 0.22mg/l). However spatial distribution of concentrations amongst stations were significant in the wet season (R^2 = 0.66) but not significant during the dry season (R^2 = 0.16) Table 1. Conversely, nitrate concentrations were relatively higher in the dry season (0.53 ± 0.28 - 0.60 ± 0.23mg/l) than during the wet season (0.33 ± 0.19 - 0.45 ± 0.51mg/l) and difference amongst stations were not significant for wet (R^2 = 0.01) and dry season (R^2 = 0.43) Table 1.

Sulphate concentrations did not demonstrate any defined spatial distribution pattern within the seasons but wet season concentrations (1.36 ± 0.76 - 57.51 ± 38.72mg/l) were observably higher than that of dry season (1.69 ± 1.58 - 21.90 ± 24.24 mg/l). However, the distribution of concentrations for dry season amongst the stations was significant (R^2 = 0.89) but wet season distribution was not significant (R^2 = 0.01) Table 1.

Amongst the nutrient variables phosphate had the highest concentrations and values increased down stream especially during the wet season (Table 1). In addition, wet season concentrations (3.9 ± 2.4 - 60.25 ± 59.35 mg/l) were higher than values observed for dry season (8.80 ± 1.65 - 10.25 ± 8.90 mg/l) and the variations amongst the stations for wet (R^2 = 0.76) and dry (R^2 = 0.95) seasons were significant.

The microbial properties defined by total coliform concentrations were relatively higher in the wet season (85.43 ± 23.78 – 299.51 ± 68.42cfu/100ml) than during the dry season (78.69 ± 34.12 – 210.63 ± 98.57cfu/100ml). The spatial distribution of concentrations amongst the zones for both seasons demonstrated significant positive relationship with the wet season (R^2 = 0.83) having closer affinity than the dry season (R^2 = 0.78) The faecal coliform concentrations demonstrated similar increasing concentration down stream and concentrations were higher in the wet season (28.66 ± 6.99 – 100. 56 ± 20.12 cfu/100ml) than during the dry (26.23 ± 7.58 – 70.21 ± 21.90cfu/100ml) with affinity between zones being significant for both season

3.3 Miniokoro

Temperature values as characteristics of equatorial tropical latitude were high for both dry (26.84 + 1.04 - 30.33 ± 1.12°C) and wet (26.22 ± 1.42 - 29.25 ± 1.40°C) seasons with dry season values being relatively higher than in the wet season. The values also increased slightly down stream (Table 1). Regression analysis indicated that dry and wet season distributions between the locations were positively significant with affinity between the stations in the dry (R^2 - 0.98) than in the wet (R^2 = 0.97). pH was acidic and values were almost uniform for dry (5.9 ± 0.54-6.57 ± 0.41) and wet (6.0 ± 0.41 - 6.35 ± 0.45) seasons(Table 1). The distribution amongst the stations were not significant for both seasons but dry season values (R^2 = 0.46) demonstrated closer affinity between stations than during the wet season (R^2 = 0.23).

Carbon dioxide concentration a measure of water acidity was considerably high with values relatively higher in the wet season (25.23 ± 6.23 - 39.67 ± 26.97mg/l) than in the dry season (18.57 ± 5.50 - 31.75 ± 12.28mg/l). The distribution of values amongst the stations was not significant in the dry season (R^2 = 0.16) but significant in the wet season (R^2 = 0.69) Table 1.

Conductivity values increased consistently down stream for both seasons and dry season (33.34 ± 7.34 - 1831.67 ± 1223.84 µS/cm) values were higher than wet season (35.72 ± 16.22 - 1053.57 ±1205. 89 µS/cm). Similarly alkalinity values increased down stream with dry season (7.17 ± 1.87 - 31.84 ± 8.31mg/l) concentrations being higher than that of wet season (7.0 ± 2.56 - 23.86 ± 10.31mg/l) Table 1.

Chloride concentrations increased down stream by several magnitudes as was observed for alkalinity and conductivity. However, wet season (1.0 ± 0.65 - 314.66 ± 133.93mg/l)concentrations were higher than dry season (1.07 ± 0.74 - 192.48 ± 167.27mg/l) and distribution amongst the stations were similar for wet (R^2 = 0.76) and dry (R^2 = 0.77)seasons were significant . Hardness concentrations were higher in the dry season (10.88 ± 9.88 - 161.20 ± 80.45mg/l) than in the wet (19.06 ± 18.4 - 137.62 ± 86.91mg/l). The relationship between the stations indicated significance between the stations for both seasons but dry season (R^2 = 0.86) had closer affinity between the stations than in the wet season (R^2 = 0.76)

Dissolved oxygen concentrations were generally high and increased exponentially from upstream to the down stream for dry and wet seasons. Concentrations were slightly higher in the dry season than in the wet season (2.56 ± 0.88 - 5.12 ± 1.55mg/l) and distribution for both dry(R^2 = 0.80) and wet (R^2 = 0.79) seasons demonstrated similar close affinity between station (Table 1).

Biochemical oxygen demand followed a similar sequence of increased concentrations down stream relatively higher concentration being observed in the dry season (23.28 ± 3.59 - 33.85 ± 5.85mg/l)than in the wet (12.92± 4.67 - 22.66 ± 5.63mg/l) Table 1.

Generally nutrient concentrations are low and amongst the nutrient variables only Sulphate demonstrated increasing concentrations from up to down stream. Others such as Phosphate, and Ammonia, had higher concentrations upstream than in other stations. Sulphate had the highest concentrations amongst the nutrient variables with dry season (0.91 ± 0.2 - 45.53 ± 29.30mg/l) concentrations being higher than the wet season (0.92 ± 0.19 - 34.25 ± 21.78mg/l) concentrations and distribution of concentrations amongst the stations for both season were significant (R^2 = 0.75) Table 1.

Nitrate concentrations for dry and wet seasons, were 0.55 ± 0.24 - 0.66 + 0.28mg/l and 0.35 ± 0.16 - 0.49 ± 0.22mg/l respectively. The differences in distribution for wet and dry seasons were not significant with wet season (R^2 = 0.22) demonstrating closer affinity between the stations than the dry season (R^2 = 0.09). Ammonia concentrations were higher in the dry season (0.42 ± 0.5 - 0.91 ± 0.39mg/l) than in wet season (0.35 ± 0.16 - 0.49 ± 0.22mg/l) with the middle reach stations having the highest concentrations for both seasons. The relationship between the stations for wet (R^2 = 0.89) and dry (R^2 = 0.99) seasons where significant with dry season having closer affinity than the wet season. The differences in phosphate concentrations for dry (0.12 ± 0.09 - 0.2 ± 0.26mg/l) and wet season (0.10 ± 0.38 ± 0.29mg/l) seasons were not remarkable but the affinity between the stations were more in the wet season (R^2 = 0.95) than in the dry season (R^2 = 0.50)

As was observed in the other stream systems total coliform concentrations recorded higher counts during the wet season (302.33 ± 52.18 – 588.77 ± 96.42cfu/100ml) than in the dry (235.12 ± 45.23 – 466.81 ± 56.41cfu/100ml) and spatial distribution of concentrations amongst the three zones for both wet (R^2=0.91) and dry(R^2=0.94) seasons were significant(Table 1). The faecal coliform count followed the same increasing concentration pattern down stream in dry season with somewhat different order in the wet season but wet season (201.45± 15.34 – 197.56 ± 28.35cfu/100ml) concentrations being higher than those of dry season (78.37 ± 10.05 – 155.60 ± 12.56 cfu/100ml). In spite of the relative high values recorded in the wet season differences between the zones were not significant (R^2 = 0.02) but dry season distribution were significant(R^2 = 0.94) Table 1.

3.4 Miniweja

Surface water temperatures were high with dry season (28.13±0.98 – 30.58±1.49°C) values being relatively higher than in the wet season (26.72±1.13 -28.29±2.49°C) and temperature tended to increase down stream for both seasons (Table 1). Dry season values (R^2 = 0.87) amongst the stations displayed closer affinity than during the wet season (R^2 = 0.76). pH was slightly acidic for wet(6.25 ± 0.27- 6.37 ± 0.34) and dry (6.24 ± 0.35 - 6.58) seasons and differences between stations were significant with wet season(R^2 = 0.95) demonstrating closer affinity between stations than the dry season (R^2 = 0.80) Table 1. Carbon dioxide concentrations were higher in wet season (36.74 ± 17.07 - 40.88 ± 13.37mg/l) than during the dry season (26.39 + 4.63 - 35.10 + 9.59mg/l) and distribution of concentrations between the stations showed closer affinity in the wet season (R^2 = 0.91) than in the dry season (R^2 = 0.89). Surface water alkalinity generally increased down stream and ranged from 11.84 ± 2.86 - 32.50 ± 23.65mg/l and 12.07 ±3.22 - 24.72 ± 10.88mg/l for dry and wet seasons respectively (Table 1). The relationships between the stations were positively significant with stations in the wet season (R^2 = 0.;96) having closer affinity than in the dry season (R^2 = 0.90). Similarly conductivity values were exceptionally high and increased down stream with higher concentrations occurring during the dry season (2263.85 ± 2433.75 - 17190.85 ± 16075.35µS/cm) than at the wet period (543 ± 1196.95- 7888.60 ± 9742.30µS/cm) Table 1. Affinity between stations was significant for wet (R^2 = 0.93) and dry (R^2 = 0.93) season. Hardness concentrations were high and spatial and seasonal concentrations pattern of increasing values down stream and higher concentrations in the dry season (333.12 ± 335.97 - 1438.72 ± 1367.80mg/l) against the wet season (183.41 ± 287.88 - 1380.35 ± 1575mg/l)as was observed for conductivity. The relationships between the stations for wet (R^2 = 0.99) and dry (R^2 = 0.96) seasons were positively significant.

Dissolved oxygen concentrations for wet and dry seasons were in the ranges of 3.64 ± 1.20 - 6.44± 2.93mg/l and 3.24 ± 1.01 - 6.91 ± 3.01mg/l respectively (Table 1).. Differences between stations were significant with dry season (R^2 = 0.93) having closer affinity than wet season values (R^2 = 0.80). Similarly BOD_5 concentrations increased downstream and concentrations were relatively higher during the dry season (18.72 ± 5.74 - 25.56 + 6.58mg/l) than in the wet season (11.65 ± 5.83 - 14.62 ± 6.67mg/l) Table 1.

High chloride concentrations were observed with relatively higher concentrations in the dry season (446.03 ± 495.13 - 2708.49 ± 2391.26mg/l) than during the wet season (99.15 ± 243.18 - 1380.35 ± 2118.31mg/l) and differences between stations for wet (R^2 = 0.99) and dry (R^2 = 0.97) seasons were significant. Suphate for dry season (61.81 ± 70.84 - 603.01 ± 486.05mg/l) were higher than concentrations in the wet season (18.64 ± 42.17 - 199.91 ± 272.36mg/l) and variations amongst stations for wet (R^2 = 0.89) and dry (R^2 = 0.97) seasons were significant. Ammonia concentrations were relatively higher in the dry season (0.19 ± 0.18 - 0.45 ± 0.42mg/l) than during the wet season (0.29 ± 0.21 - 0.38 ± 0.42mg/l) and variations between stations were only significant in the wet season (R^2 = 0.55) but not significant during the dry season (R^2 = 0.22). Nitrate concentrations appeared relatively higher in the wet season than in the dry and ranged from 0.68 ±0.18 - 0.81 ± 0.31mg/l and 0.61 ± 0.27 - 0.91 ± 1.33mg/l for dry and wet seasons respectively. The affinity between stations were higher in the dry season (R^2 = 0.93) than during the wet season (R^2 = 0.50). Similarly phosphate concentrations spatially tended to increase down stream and wet season concentrations were higher than that of the dry season (0.13 ± 0.12 - 0.15 ± 0.14mg/l),seasonal differences amongst the stations were significant (R^2 = 0.99) for both seasons(Table 1).

Total coliform distributions exhibited obvious seasonal changes (Table 1) with Dry season (342.00 ± 45.34 – 533.00 ± 76.80cfu/100ml) concentrations being relatively lower than wet season concentration (621.86 ± 76.33 – 782.15 ± 95.83cfu/100ml). However the distribution of concentrations amongst the stream course was significant in dry season (R^2 = 0.98) but not significant in wet season (R^2 = 0.98). Faecal coliform recorded lower concentrations against the total coliform with similar seasonal trend such that dry season (114.00 ± 10.07 – 177.54 ± 17.06 cfu/100ml; R^2 = 0.98) concentrations were lower than that of wet season (208.63 ± 22.45 – 296.39 ± 28.18 cfu/100ml; R^2 = 0.37)

3.5 Ntawogba

surface water temperature values were generally high with mean values ranging from 26.83 ± 0.44 -27.08 ± 0.21 in wet season while dry season values ranged from 27.75 ± 0.32o -28.17 ± 0.31oC(Table 1). Spatial variation between stations demonstrated significance for both seasons with affinity between the stations being closer in the wet season ($R^2 = 0.96$) than during the dry season ($R^2 = 0.57$).

The pH was slightly acidic for both seasons and differences between the seasons were minimal and values ranged from 6.46 ± 0.16 - 6.57 ± 0.18 and 6.17 ±0.03 - 6.29± 0.05 for wet and dry seasons respectively (Table 1). Spatial differences between the study stations for wet ($R^2= 0.10$) and dry ($R^2=0.10$) seasons were not significant. Carbon dioxide concentrations for wet and dry seasons stood at 25.82 ± 11.88 - 38.1 ± 19.52mg/l and 11.79 ± 4.49 - 24.42 ± 16.48mg/l and differences amongst the stations were significant demonstrating more affinity in the dry season ($R^2 = 0.69$) than during the wet season ($R^2 = 0.67$).

Conductivity values were high, ranging from 188.25 +15.17 - 265.0 ±25µS/cm in the wet season and 251.67 ± 17.69 - 375.08µS/cm in dry season (Table 1). There were relative differences on spatial basis with values increasing down stream and seasonal differences amongst stations were significant with dry season ($R^2 = 0.90$) demonstrating closer affinity amongst the stations than during the wet season ($R^2 = 0.90$).

Alkalinity values for wet and dry seasons increased down stream with higher concentrations recorded in the dry (62.83 + 13.10 - 89.67 + 16.67mg/l) than during the wet season (10.08 ± 1.76 - 14.00 ± 2.25mg/l) and spatial differences between the stations demonstrated significance for wet ($R^2 = 0.96$) and dry season ($R^2 = 0.97$).

There was no clear spatial trend demonstrated in the dissolved oxygen distribution other than the fact that the highest concentrations occurred at the upper limit station for both seasons (Table 1) differences between the stations were significant ($R^2 =0.61$) while dry season differences between stations were not significant ($R^2 = 0.26$). In all, concentrations were relatively higher in the wet season (6.50 ± 0.50 - 8.42 ± 0.80 mg/l) than during the dry (5.55 ± 0.48 - 7.35 ± 0.65mg/l). BOD_5 concentrations increased almost exponentially down stream with differences in concentrations between wet and dry seasons being 13.45 ± 3.50 - 37.86 ± 8.54mg/l and 26.45 ± 9.67 - 55.25 ± 7.44mg/l respectively. The stations demonstrated similar significant differences for wet ($R^2 = 0.98$) and dry ($R^2 = 0.99$) seasons

Ammonia concentrations similarly increased downstream for wet and dry seasons and concentrations were higher in the dry season (0.85±0.14 - 2.10 ± 0.22mg/l) than during the wet season (0.41 ± 0.15 - 0.47± 0.23mg/l) Table 1. Spatially, concentrations between stations were significant during both seasons with stations having closer affinity during the wet season ($R^2 = 0.98$) than during the dry season ($R^2 = 0.57$). Sulphate concentrations were in magnitude of about two times higher in the dry (10.40 ± 2.40 - 13.69 ±3.99mg/l) than in the wet season (4.34 ± 1.60 - 5.78 + 1.36mg/l) and concentrations increased down stream during both seasons. Significant differences were observed amongst the stations for both seasons with affinity between stations being observed during the dry season ($R^2 = 0.98$) than during the wet season ($R^2 = 0.53$). Nitrate concentrations were comparably high with steady increase in concentration from upstream to down stream station. The differences between stations were significant with closer affinity being observed in the dry season ($R^2= 99$) than in the wet ($R^2= 98$). Similarly, phosphate concentrations demonstrated an increasing concentrations from upstream to the downstream limit and differences between stations were significant with closer affinity being observed in the wet season ($R^2 = 0.91$) than during the dry (R^2 = 0.81). Dry season (0.62 ± 0.09 - 0.99 ± 0.20mg/l) concentrations were higher than that of the wet season (0.41 ± 0.15 - 0.70 ± 0.23mg/l) Table 1.

4. Discussion

Generally, the stream systems maintained high temperature values for both wet and dry seasons and this is a common characteristic reported for the Niger Delta waters (RPI, 1985, NES, 2000) which are located at the equatorial latitude where temperature is consistently high all the year round. In all, a number of associations emerged with temperature such that during the wet season, a strong positive correlation between temperature and Alkalinity (r = 0.69), conductivity (r^2 =0.61), hardness (r =0.60), DO (r^2 =0.73), BOD (r^2 =0.55), So_4 (r^2 =0.61) TC (r^2 =0.76) and FC (r^2 =0.58) Table 2. Similarly, in dry season temperature had significant positive correlation with conductivity (r^2 =0.82), Hardness (r^2 =0.82), DO (r^2 =0.63), BOD (r^2 =0.72), SO4 (r^2 =0.76) Total coliform (r^2 =0.77) and faecal coliform (r^2 =0.78) but negative association was observed for dry season period between temperature and carbon dioxide (r^2 = -0.56) Table 3.

The acidity of a water body is an important factor that determines the suitability of water for various purposes, including toxicity to animals and plants. With the exception of Agbonchia stream whose ph varied from slightly acidic to neutral, the stream systems under study were slightly acidic , showing no consistent spatial and seasonal trends. It is pertinent to observe that while the general values of the water bodies may appear alright comparable to WHO (19 84)limits for potable water the values for such systems in the past had been in the range of 4.5 – 6.0 and 4.8 – 6.5 for wet and dry seasons respectively(NDBDA,1987, Igbinosa and Okoh, 2009). The present pH values are considered high for such soft acid water bodies draining forested wet land with leaf litter that impact humic acid substances that give it the low acidity. The change in pH observed which rather tended toward neutrality might be due to decreased forest floor drainage area, washing of concrete structures during storm and increasing draining of domestic effluent water to the stream.as well as influence of brackish water. pH in the wet season was observed to have significant positive correlation with PO_4 (r^2 =0.58), and negatively correlated with total coliform (r^2 =-0.61) and FC (r^2 =-0.65)Table 2 while in the dry season, pH positively correlated only with PO_4 (r^2 =0.53) and negatively correlated with CO_2 (r^2 =-0.57) Table 3.

Conductivity is a measure of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions; on their total concentration, mobility, as well as valence; and the temperature of measurement. The relationship with other parameters of note are the positively correlated with hardness (r^2 =0.97), DO (r^2 =0.65), BOD_5 (r^2 =0.58), NO_3(r^2 =0.55), SO_4 (r^2 =0.96), TC (r^2 =0.69) in the wet season but in the dry season, significant positive associations were observed between conductivity and DO (r^2 =0.60), BOD_5 (r^2 =0.64), SO_4 (r^2 =0.84), TC (r^2 =0.72) and FC (r^2 =0.72) (Table 2 and 3)

Total hardness of all the water bodies showed higher concentration in the dry season than in the wet season. this is primarily due to reduced inflow and evaporation, while the relative lower concentrations observed may be attributed to increasing inflow and dilution. However to high hardness generally observed in the water bodies may in part be associated the the concrete structure covering the path of the stream. Hardness was found to positively correlation with DO (r^2 =0.67), NO3 (r^2 =0.60), SO4 (r^2 =0.97),TC (r^2 =0.69), and FC (r^2 =0.50) in wet season but in dry season slight variation in the relationships between the attributes such as the positive correlation with DO (r^2 =0.58), BOD (r^2 =0.66), SO4 (r^2 =0.81), TC (r^2 =0.74) and FC (r^2 =0.75) Tables 2 and 3.

🟩	Negatively significant
🟨	Positively significant

Wet season														
	T °C	*pH*	*CO$_2$*	*ALKALINITY*	*CONDUCTIVITY*	*HARDNESS*	*DO*	*BOD$_5$*	*NH$_4$-N*	*NO$_3$-N*	*SO$_4^{2-}$*	*PO$_4$- P*	*Total Coliform*	*Faecal Coliform*
T °C	*1*													
pH	-0.33	1												
CO$_2$	-0.21	-0.49	1											
ALKALINITY	0.69	-0.14	-0.12	1										
CONDUCTIVITY	0.61	-0.11	-0.22	0.66	1									
HARDNESS	0.60	-0.14	-0.12	0.60	0.97	1								
DO	0.73	0.05	-0.03	0.54	0.65	0.67	1							
BOD$_5$	0.55	-0.13	-0.21	0.81	0.58	0.47	0.42	1						
NH$_4$-N	0.31	-0.06	0.07	0.82	0.20	0.14	0.21	0.63	1					
NO$_3$-N	0.41	-0.24	0.23	0.53	0.55	0.60	0.43	0.26	0.23	1				
SO$_4$2-	0.61	-0.05	-0.15	0.58	0.96	0.97	0.72	0.43	0.09	0.58	1			
PO$_4$- P	-0.32	0.58	-0.12	-0.25	-0.14	-0.13	0.06	0.07	-0.21	-0.29	-0.15	1		
Total Coliform	0.76	-0.61	0.25	0.70	0.69	0.69	0.56	0.58	0.35	0.68	0.65	-0.32	1	
Faecal Coliform	0.58	-0.65	0.49	0.59	0.44	0.50	0.37	0.38	0.41	0.58	0.45	-0.35	0.87	1

Table 2. The correlation coefficient between the physicochemical and biological variables in the wet season

	T °C	*pH*	*CO_2*	*ALKALINITY*	*CONDUCTIVITY*	*HARDNESS*	*DO*	*BOD_5*	*NH_4-N*	*NO_3-N*	*SO_4^{2}-*	*PO_4- P*	*Total Coliform*	*Faecal Coliform*
T °C	1													
pH	0.25	1												
CO_2	-0.56	-0.57	1											
ALKALINITY	0.40	0.46	-0.67	1										
CONDUCTIVITY	0.82	0.33	-0.43	0.25	1									
HARDNESS	0.82	0.34	-0.44	0.28	1.00	1								
DO	0.63	0.28	-0.19	-0.17	0.60	0.58	1							
BOD_5	0.72	0.17	-0.63	0.56	0.64	0.66	0.22	1						
NH_4-N	0.16	0.38	-0.57	0.95	0.11	0.14	-0.40	0.47	1					
NO_3-N	0.27	-0.31	0.23	-0.30	0.06	0.05	0.52	-0.04	-0.41	1				
$SO_4$2-	0.76	0.18	-0.24	0.13	0.84	0.81	0.65	0.29	-0.08	0.21	1			
PO_4- P	-0.42	0.53	0.02	-0.26	-0.26	-0.26	0.21	-0.49	-0.23	-0.14	-0.24	1		
Total Coliform	0.77	0.26	-0.59	0.57	0.72	0.74	0.18	0.88	0.49	-0.01	0.47	-0.55	1	
Faecal Coliform	0.78	0.29	-0.64	0.67	0.72	0.75	0.16	0.90	0.58	-0.06	0.47	-0.55	0.99	1

Table 3. The correlation coefficient between the physicochemical and biological variables in the dry season

Dissolved oxygen is one of the most vital factors in assessing stream quality. Its deficiency directly affects the ecosystem of a stream due to several factors which include physical, chemical, biological and microbiological processes. DO is needed to support biological life in aquatic systems. The levels observed for the study streams are so low that they may not sufficiently support aquatic life including fish. This objectionable low concentration occurred at both seasons, may be associated with the municipal discharges and the attendant organic load and utilization in bacterial decomposition of organic matter. DO in wet season correlated significant with SO_4 (r^2 =0.72), and TC (r^2 =0.56) and in the dry season such associations were observed with NO_3 (r^2 =0.52) and So_4 (r^2 =0.65) Tables 2 and 3.

Biological oxygen demand, being a measure of the oxygen in the water that is required by the aerobic organisms and the biodegradation of organic materials exerts oxygen pressure in the water and increases the biochemical oxygen demand (Abida, 2008). Streams with low BOD_5 have low nutrient levels; and this may account for the general low nutrient status of the stream in most cases.

The increased concentration of BOD_5 implies that oxygen is swiftly depleted in the streams. The consequences of high BOD_5 concentrations are the same as those for low dissolved oxygen: thus organisms are prone to stress, suffocate, and possibly death. In wet season, BOD_5 correlated with NH_4 (r^2 =0.63)and TC (r^2 =0.58) while in dry season the relationships that emerged were significant positive correlation with TC (r^2 =0.88) and Fc (r^2 =0.90) Tables 2 and 3.

Ammonia, a transitional nutrient, generally recorded higher values in the dry season than in the wet season. The distribution of concentration followed a pattern of Nta Wogba > Minchida > ,Minweja > Minikoro > Agboncha in the dry season and in the wet season a slight shift was observed such that the concentration sequence being Nta Wogba > Miniokoro> Minichida > Miniweja > Agboncha

Similarly the same seasonal differences were observed in the distribution of nitrate with higher concentrations in the dry season than in the wet season and the distribution of concentrations being in the decreasing order of Miniweja > miniokoro > Agboncha > Nta wogba > Minichida and Minweja > Ntawogba > Miniokoro >Minichida =Agboncha for dry and wet season periods respectively

The sulphate was the highest of all the nutrients in the different stream and it is considered major composition of seawater following the role of municipal and industrial wastes on sulphate addition to of surface water bodies. The distribution of sulphate concentrations followed a decreasing order of Miniweja stream > Ntawogba stream > Miniokoro stream > Aboncha stream > Minichida stream and Miniweja stream > Ntawogba stream > Agbonchia stream > Miniokoro stream > Minichida stream for dry and wet seasons. However, it is pertinent to note that values observed for Miniweja and Ntawogba were by hundreds of magnitude higher than values observed in the other stream systems

Phosphates as with nitrates are important in assessing the potential biological productivity of surface waters. Increasing concentration of phosphorus and nitrogen compounds in streams or rivers may lead to eutrophication. In this study higher concentrations were recorded in the wet season than in the dry seasons for all the streams and concentrations were considered normal for all the streams except at Agboncha stream in which the distribution of concentration followed a declining order of Agboncha stream > Nta wogba stream > Miniokoro stream >Miniweja stream > Minichida stream and Agboncha stream > Ntawogba stream > Miniokoro stream > Miniweja stream > Minichida stream for dry and wet seasons respectively. The high phosphate value in Agboncha stream may be related in part to Abattoir discharges and petrochemical waste discharges into the system.

The comparison of the variables for the streams using 2 -way Analysis of variance (ANOVA) for the upper limit stations in the wet season demonstrated non significance between the variables (ANOVA = 2.06 , < F ($2.08_{(0.05)}$)) and between streams (ANOVA = 1.88 < F = $2.61_{(0.05)}$)) Table 4. The middle reach limits of the streams also demonstrated non significance for the variables (ANOVA= 1.15 < F = $2.08_{(0.05)}$)) and between streams (ANOVA = 1.34 < F = $2.61_{(0.05)}$) Table 4. The downstream limits demonstrated a contrary pattern with significance been observed for the variables (ANOVA = 3.06 > F = $2.15_{(0.05)}$)) but stream differences were also not significant (ANOVA = 1.33 < F = 2.63 $_{(0.05)}$) Table 4.

Upstream limits

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Variables	97035.61	10	9703.561	2.06	0.05	2.08
Water bodies	35111.77	4	8777.944	1.879257	0.13	2.61
Error	186838.6	40	4670.966			
Total	318986	54				

Middle Reach limits

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Variables	7180969	10	718096.9	1.15	0.35	2.08
Streams	3346749	4	836687.2	1.34	0.27	2.61
Error	24964554	40	624113.9			
Total	35492272	54				

Down Stream limits

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Variables	87980538	9	9775615	3.06	0.01	2.15
Stream	16958067	4	4239517	1.325206	0.28	2.63
Error	1.15E+08	36	3199139			
Total	2.2E+08	49				

Table 4. The 2 way Analysis of variance comparing the variables and the streams at different limits in the wet season

Similar trend was observed in the dry season with differences between variables (ANOVA = 1.38 < F = 2.08 $_{(0.05)}$) and the streams (ANOVA = 1.40 < F = $2.61_{(0.05)}$) for the upper limit stations were not significant. The middle reach limits also demonstrated same pattern as observed with the upper limit with differences between the variables (ANOVA = 1.30 < F = 2.08 $_{(0.05)}$) and the streams (ANOVA = 1.25 < F = $2.61_{(0.05)}$) not being significant. The down stream limit demonstrated that the differences between the variable (ANOVA = 2.96 < F = $2.08_{(0.05)}$) were significant but differences between the streams (ANOVA = 1.24 < F = $2.61_{(0.05)}$) were not significant (Table 5).

Upstream limits

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Parameters	1185660	10	118566	1.38	0.23	2.08
Streams	482331.8	4	120582.9	1.40	0.25	2.61
Error	3441178	40	86029.46			
Total	5109170	54				

Middle stream limits

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Parameters	38261014	10	3826101	1.30	0.27	2.08
Streams	14808576	4	3702144	1.25	0.30	2.61
Error	1.18E+08	40	2950478			
Total	1.71E+08	54				

Down stream limits

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Parameters	3.63E+08	10	36281955	2.96	0.01	2.08
Streams	60805895	4	15201474	1.24	0.31	2.61
Error	4.91E+08	40	12271158			
Total	9.14E+08	54				

Table 5. The 2 way Analysis of variance comparing the variables and the streams at different limits in the dry season

The five streams have similar physiochemical characteristics apparently because they drain from analogous freshwater systems upstream through the stretch of the city into brackish water systems of the Bonny estuary downstream. The study shows that conductivity values are only higher in dry season in Miniweja out of other streams where the values are generally lower in dry season. The reason could be as a result of the study area of Miniweja being more influenced by brackish water than in any other stream. Minichinda, Nta wogba, Miniokoro and Agboncha streams appear to have more influence of the municipal waste water during wet season.

The similarities in characteristics of the streams are further demonstrated by apparently similar pH values obtained. Naturally, the upstream stations are expected to have much more acidic pH values as a result of vegetation and humic substance released into the forest systems (RPI, 1985, Chindah *et. al.*, 1999, Chindah, 2003, Obunwo, *et. al.*, 2004). Then the pH value increases gradually to become more alkaline as the down stream stations of are approached to the influence of brackish water (RPI, 1985, NDES, 2000, NDDC, 2004 and Izonfuo *et. al.*, 2005). However, in the study, the pH values are apparently uniform with only slight spatial differences indicating that the wastes along the course of the stream have altered the characteristics (Brion and Billen (2000).

Nutrient concentrations are generally low except at the down stream of Miniweja stream where phosphate concentrations were very high. The reason for the general low nutrient concentrationin-spite of the organic load received by the systems may be due to both the high temperature and microbial properties of the water body. Organisms in tropical water bodies are known to quickly use up the nutrients under high temperature condition (Chindah and Braide, 2004 and Chindah *et. al.*, 2005).

This effect is also observed in other parameters. For example, the general low dissolved oxygen concentrations in most streams and the relatively higher values of oxygen recorded in the upstream stations comparative to the mid and down stream stations implies the depletion of oxygen along the water course as it flows down stream. This may suggest that the more waste inputs are received by the streams the more its dissolved oxygen concentration declines. Conversely the BOD_5 values are very high and generally increased down stream. This supports the contention that the increased waste load into the system degrades the water quality as the BOD_5 values far exceed concentrations reported in the baseline studies of some of these streams (NDBDA 1987, and Ogan 1988) Therefore it is our contention that the low oxygen concentrations recorded and the high BOD_5 values for all the streams are strong evidence to suggest the impact of organic load introduced from municipal waste into the streams (Rim-Rukeh *et. al.*, 2007, Hill *et. al.*, 2005 and Chen, 2010). Similarly other indices implicating municipal waste discharges on the stream systems are the high total coliform and faecal coliform concentrations observed in the water bodies which are below concentrations recorded in most of the systems in the past studies (Amadi *et. al.*, 1997, Odokuma and Okpokwasili, 1997 and Ogan 1988). The present total coliform and faecal coliform concentrations indicate the seriousness of the impact of municipal waste water on receiving surface waters and the health hazards implication to ignorant users especially children (Braide *et. al.*, 2004, Okoh *et. al.*, 2005 and 2007). The study shows that the rapid growth of Port Harcourt and associated municipal wastes introduced into the five main steams have caused the deterioration of the water quality of the streams and therefore presents the need for a better waste management system (Chen, 2010).

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15

Impact of Municipal Waste Water on Growth and Nutrition of Afforested *Pinus eldarica* Stands

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1. Introduction

As a whole, water is a most important source for plantations particularly in the dry regions (Mosadegh, 1999). In other hand, wastewater can be used to cover the needs of urban and rural areas and parks as well as industrial complexes to develop green space and to reduce air pollution (Al-Jamal *et al.*, 2000; Singh and Bhati, 2005; Sharma, *et al.*, 2007). In reality, wastewater except the water resource for irrigating the plantations is an enormous nutrient source, too (Meli *et al.*, 2002; Rattan *et al.*, 2005). Of course, establishment of trees plantation for waste water irrigation has been a common practice for many years. The practice not only defers ecological degradation by the pollutants in the soil, because trees are long-living organisms which can take up trace elements from the soil, water or air and retain them for a long time (Madejo´n *et al.*, 2006). But it also creates opportunities for commercial biomass production and sequestration of excess minerals in the plant system (Sharma and Ashwath, 2006). Therefore, the use of waste water in growing woodlots is a viable option for the economic disposal of waste water (Neilson *et al.*, 1989). Moreover, waste water from municipal origin is rich in organic matter and also contains appreciable amounts of macro and micro-nutrients (Gupta *et al.*, 1998). Accordingly nutrients levels of soil are expected to improve considerably using continuous irrigation with municipal waste water (Ramirez-Fuentes *et al.*, 2002; Rattan, *et al.*, 2005). Apart from this, in the case of the utilization of wastewater mixed with harmful heavy metals lead to decrease the toxicity, through a developed rooting system in plantations (Karpiscak *et al.*, 1996) and as such, play the important and fundamental role for the environmental protection (Cromer *et al.*, 1987; Stewart *et al.*, 1990). However, this can not be ignored that the use of wastewater for irrigation purposes might damage the ecosystem because the high toxic concentration and heavy metals (Gupta *et al.*, 1998; Brar *et al.*, 2000; Yadav *et al.*, 2002). The accumulation of heavy metals in soil is related to pH, texture and cation exchange capacity of soil (Datta *et al.*, 2000). Therefore, decision about the application of wastewater should be made based on the views of specialties of water, soil, plant and environment of every location (Nagshinepour, 1998).

Iran is a part of arid regions in the world being encountered acute crises owing to the increased population and need of water resources (Tabatabaei, 1998). It is noteworthy saying that thousands liters of domestic, industrial and hospital effluents are daily flowing from Tehran metropolitan area and influence the underground water resources. In the same way, 80 percent of the useful water of the citizens in Tehran is also transformed as municipal effluent (Tajrishi, 1998). On the other side, unplanned expansion and air pollution of Tehran make it unavoidable to increase the green space. In reality, urban green space and green belt around the city can play an effective role in air purification and climate health. Since the lack of water is a limiting factor for development of green space, therefore municipal effluent may be suitable (Torabian and Hashemi, 1999).

Till now inside the country several researches have been conducted about effect of municipal effluent on soil and agricultural crops, but not on softwoods. The objective of this study was to investigate the effects of the 15 years municipal waste water application on the growth of *Pinus eldarica* Medw. trees and the minerals accumulation in the trees needles.

2. Materials and methods

The study site is an abandoned agriculture site located in Shahr-e Rey, 5 Km south of Tehran-Iran (Latitude 35° 37' N, Longitude 51° 23' E, 1005 m above sea level). The climate of the site is semi-arid with mild-cold winters and 7 months (Mid April-Mid November) dry season (Fig. 1). Average annual rainfall and average annual temperature are 232 mm and 13.3° C, respectively. The highest rainfall appears in March and the lowest in August. The warmest month occurs in August and the coldest in January. Experiment was conducted at two 4 hectare even-aged (15 years) artificial stand of *Pinus eldarica* Medw. The first stand was irrigated with municipal waste water and the second with well water since plantation. The irrigation was applied daily based on tree water-use and the potential evapo-transpiration, which varied seasonally in response to the climate. The soils of both fields were clay-loam with 32.5% clay, 34.12% silt and 33.38% sand in the field irrigated with municipal waste water and 28.52% clay, 36% silt and 35.48% sand in the field irrigated with well water

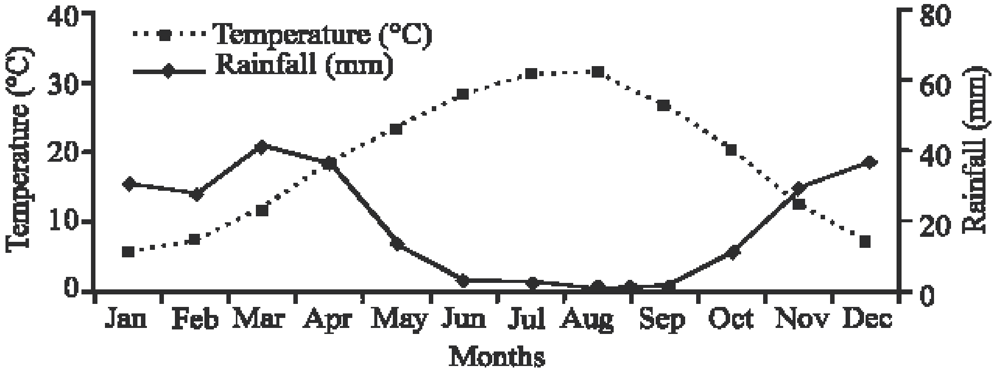

Fig. 1. Embrothermic curve of the study site

The study was established in October 2006. Data was collected using technique of systematic random sampling (Jayaraman, 2000) with 4 replications in either of both fields. Therefore, four plots were identified in each field of irrigated with municipal waste water and well water. Plots were 30 m × 30 m, with tree spacing of 3 m × 4 m (833/ha). In each plot, diameter at breast height (d.b.h.), total height, crown length and crown diameter of total trees were measured and basal area computed. Standing volume of each tree was determined by using form factor (~0.5) and formula $V = 0.4 \times D^2 \times H$, made by Zobeyri

(1994). Where, D is diameter at breast height (d.b.h.), H is total height and V is standing volume.

In each plot, four trees were selected and at the end of growing season needle samples of *P. eldarica* trees taken from the top of crown and the part affected by sunlight (Letacon, 1969; Habibi Kaseb, 1992). This collection provided 16 needle samples in each treatment. At the end of the sampling, one representative needle sample from each plot (by mixing of four samples of each plot) was taken (due to decreasing of samples quantity for chemical analysis). Municipal waste water and well water were sampled daily (3 days in each month) from early June to late November, at three times per day (morning, noon and evening) to make a composite sample of each day.

Water samples were brought to the laboratory in resistant plastic bottles to avoid adherence to the container wall. They were filtered through 42 mm filter paper and stored at 4 °C to minimize microbial decomposition of solids (Yadav *et al.*, 2002; Bhati and Singh, 2003). Several parameters were measured separately, pH and EC by the procedure described using OMA (1990), NH_4-N, NO_3-N, PO_4-P, K, Ca, Mg and Na as per the method given by APHA (1992) and Yadav *et al.* (2002).

Fresh weight of some needles from each treatment was recorded immediately after harvest. Dry weight was recorded after oven drying of needles for 72 h at 80 °C (Bhati and Singh, 2003). Samples of needle were washed using tap water, rinsed with distilled water, oven dried at 80 °C for 24 h (Singh and Bhati, 2005), ground in a stainless steel mill and retained for mineral analysis. For determination of macro and micro-nutrients exception P and N, the needle samples were wet digested as per Jackson (1973) and estimated using an Atomic Absorption Spectrophotometer (AAS). Measurement of P content was performed after a wet digestion using UV–VIS spectrophotometer at 450 nm (Singh and Bhati, 2005). The N content of needle samples digested in concentrate sulfuric acid was determined by the Kjeldahl method (Bhati and Singh, 2003; Bozkurt and Yarilga, 2003).

Average growth parameters and needle nutrients of two irrigation treatments (T_1: irrigation by municipal waste water; T_2: irrigation by well water) were compared using independent-samples t-test. The variations in characteristics of municipal waste water and well water were firstly tested for normality using Shapiro-Wilk's test and then by independent-samples t-test. All the data were analyzed using the SPSS statistical package.

3. Results and discussion

3.1 Waste water and well water

Results indicated that the waters were alkaline in reaction (Table 1). The pH of the municipal waste water in various months ranged from 7.51 to 7.75 and for well water 6.69 to 7.62. Based on results of Patel *et al.* (2004), in our examination the tolerance limit of pH for irrigation ranged from 6.0 to 9.0. The electrical conductivity (EC) of municipal waste water ranged from 1.78 to 2.12 dS m^{-1} with the greatest value detected in August. Average EC of municipal waste water (mean of 18 samples) exceeded 1 dS m^{-1} (1.91 dS m^{-1}) indicating the waste water was saline in nature (Rattan *et al.*, 2005). The pH and EC of the municipal waste water were greater than those of the well water. The concentration of all the nutrient elements was higher in municipal waste water, with NO_3-N content (1.63 mg l^{-1}) being 6.8 times the content in well water (0.24 mg l^{-1}). The content of NH_4-N in municipal waste water (9.05 mg l^{-1}) was also 4.2 times the content in well water (2.15 mg l^{-1}). On average, available content of PO_4-P, K^+, Ca^{2+}, Mg^{2+}, Na^+ in municipal waste water were greater compared to those in the well water. The most nutrients concentration of municipal waste water were reduced in autumn and increased in summer because of high temperature and evaporation losses of water (Singh and Bhati, 2005).

Although municipal waste water elevated significantly ($P < 0.01$) in all values compared to well water, but the analysis showed that pH, EC, NO_3-N, PO_4-P, K^+, Na^+ of well water samples were within the limits as per the standard prescribed for land disposal and should not pose any serious hazard according to threshold values of WHO (Hach, 2002). However, the contents of NH_4-N and Ca^{2+} of municipal waste water and well water and Mg^{2+} of municipal waste water were on the higher side (Table 1).

Parameters	Municipal waste water		Well water		WHO[*]
	Range (Min.-Max.)	Mean ± SE	Range (Min.-Max.)	Mean ± SE	
pH	7.51 - 7.75	7.63 ± 0.01 [a]	6.69 - 7.62	7.32 ± 0.05 [b]	6.5 - 8.5
EC (dS m^{-1})	1.78 - 2.12	1.91 ± 0.02 [a]	0.54 - 0.67	0.590 ± 0.008 [b]	3
NH_4-N (mg l^{-1})	8.1 - 10.24	9.05 ± 0.11 [a]	1.83 - 2.49	2.15 ± 0.19 [b]	1.5
NO_3-N (mg l^{-1})	1.58 - 1.89	1.63 ± 0.09 [a]	0.19 - 0.33	0.24 ± 0.08 [b]	3
PO_4-P (mg l^{-1})	11.45 -14.13	12.69 ± 0.16 [a]	4.62 - 5.64	5.03 ± 0.01 [b]	---------
K (mg l^{-1})	33.06 - 46.31	39.93 ± 0.83 [a]	17.48 - 22.75	19.72 ± 0.36 [b]	---------
Ca (mg l^{-1})	235.54 - 296.20	255.22 ± 4.57 [a]	66.70-101.57	96.77 ± 1.26 [b]	75
Mg (mg l^{-1})	100.9 - 124	109.85 ± 1.83 [a]	28.9 - 42	35.22 ± 0.79 [b]	50
Na (mg l^{-1})	135.90 - 150.22	140.45 ± 0.20 [a]	30.18 - 41.03	35.18 ± 0.13 [b]	200

Different superscripts in row indicate significant ($P < 0.01$) difference. Values are mean of eighteen replications (3 days × 6 months) with ± SE; [*] World Health Organization (WHO): Hach, 2002

Table 1. Characteristics of municipal waste water and well water

3.2 Tree growth

Irrigation with municipal waste water for 15 years produced the largest trees in this treatment. The most frequent trees were found at diameter class of 20 cm and 14 cm, respectively grown on field irrigated with municipal waste water and well water (Fig. 2). In fact, tree growth was greater ($P < 0.01$) in the field irrigated using municipal waste water than in plots irrigated with well water, as indicated by the 17.95 ± 1.33 cm diameter at breast height, 10.04 ± 0.15 m height, 8 ± 0.27 m crown length, 2.53 ± 0.17 m crown average diameter, 264.20 ± 30.02 cm^2 basal area and 0.139 ± 0.013 m^3 standing volume of the trees in waste water irrigated field (Table 2). Similarly, an increase in the growth of olive (*Olea europaea*) trees due to irrigation with municipal waste water has been reported by Aghabarati *et al.* (2008). The study of Stewart *et al.* (1990) also suggested that the addition of municipal waste water on *Eucalyptus grandis* has been resulted in a doubling of growth rate when compared to *E. grandis* grown in a rain fed site in four years.

The increased growth may be linked to sufficient availability of water and better status of nutrients in soil (Larchevêque *et al.*, 2006). Since municipal waste water contains plant nutrients and organic matter, it may improve the properties of soil for increase in growth and biomass production (Guo *et al.*, 2002; Egiarte *et al.*, 2005; Lopez *et al.*, 2006). The increase in growth indicates that waste water application influenced the physiological processes, facilitated early needle initiation and resulted in a net increase in the number of needles. An increase in needles could have captured more solar energy for metabolic use, fixed more CO_2, and produced greater photosynthesis, and growth. This hypothesis is supported by Ceulemans *et al.* (1993) and Myers *et al.* (1996).

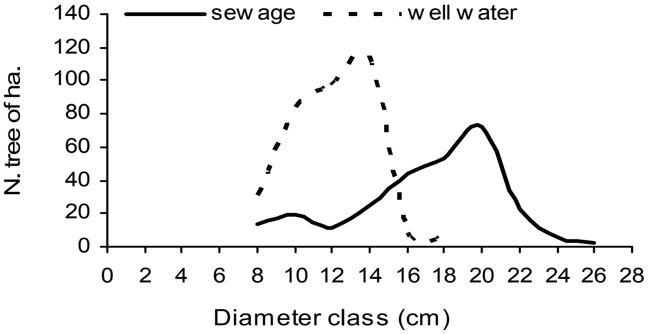

Fig. 2. Distribution of diameter classes for *P. eldarica* trees in two irrigation types

Irrigation type	Diameter at breast height (cm)	Height (m)	Crown length (m)	Crown diameter (m)	Basal area (cm^2)	Standing volume (m^3)
Trees irrigated with waste water	17.95 ^{a} (1.33)	10.04 ^{a} (0.15)	8.0 ^{a} (0.27)	2.53 ^{a} (0.17)	264.20 ^{a} (30.02)	0.139 ^{a} (0.013)
Trees irrigated with well water	13.50 ^{b} (0.5)	9.02 ^{b} (0.10)	7.3 ^{b} (0.12)	1.90 ^{b} (0.20)	135.0 ^{b} (20.5)	0.65 ^{b} (0.09)

-Different superscripts in column indicate significant difference of each tree attribute between two irrigation types.

-Values in parenthesis are ± SE.

Table 2. Effect of municipal waste water and well water on growth of *P. eldarica* trees

3.3 Mineral composition of needles

The application of municipal waste water significantly increased the macro-elements (N, P, K, Ca, Mg, Na concentration of *P. eldarica* trees needle as compared with well water (Table

3). Increases in minerals concentration may have been due to the effect of nutrients addition through municipal waste water (Meli *et al.*, 2002). This result is in agreement with Singh and Bhati (2005) and Aghabarati *et al.* (2008), whereas a substantially greater above-mentioned minerals concentration were observed in leaf of *Dalbergia sissoo* seedlings and *Olea europaea* trees irrigated with municipal waste water compared to control. However, Guo *et al.* (2002) and Aghabarati *et al.* (2008) had also suggested that a decrease of Mg and Ca, and no difference of Na concentration in leaf of eucalypt and olive tree were treated by municipal waste water. In fact, quantity of nutrients absorption using plant depends upon the total quantity of the nutrients applied through waste water application, soil properties and type of plant (Bozkurt and Yarilga, 2003). The minerals concentration of needle may be ranked from greatest to least as N > Ca > K > Mg > P > Na.

	N	P	K	Ca	Mg	Na
	------------------------------ gr kg $^{-1}$ ------------------------------					
Soil treated with T_1	16. 41^{a} (0.27)	0.865 ^{a} (0.058)	5.79 ^{a} (0.50)	6.08 ^{a} (0.27)	1.51^{a} (0.12)	0.320 ^{a} (0.027)
Soil treated with T_2	15.47 ^{b} (0.35)	0.710 ^{b} (0.014)	4.49 ^{b} (0.42)	4.64 ^{b} (0.26)	1.28 ^{b} (0.11)	0.198 ^{b} (0.034)
p-value	<0.01	<0.05	<0.01	<0.01	<0.05	<0.01
Range *	5-30	1-5	3-30	10-40	1-7	------

Abbreviations: T_1: municipal waste water; T_2: well water; values are mean of four replications with ± SD in parentheses; different superscripts in column indicates significant difference between T_1 and T_2; * Salardini (1992)

Table 3. Mineral composition of *P. eldarica* trees needle by affected by municipal waste water and well water

4. Conclusion

Our study displayed that all growth parameters measured in *P. eldarica* trees were statistically greater in effluent-irrigated area than in well-watered area. As a whole, the use of municipal effluent in irrigations can be an overflowing resource from the nutrient elements required for plants (Yadav *et al.*, 2002; Mapanda *et al.*, 2005; Toze, 2006). As a matter of fact, high nutrient concentrations in effluent, compared to those in well water, cause the nutrient accumulation in the soil (Stewart and Flinn, 1984; Phillips *et al.*, 1986; Stewart *et al.*, 1990; Keller *et al.*, 2002; Selivanovskaya *et al.*, 2002; Emongor and Ramolemana, 2004) and makes easy the access of plants to the high nutrient concentration (macro and micro elements) and increases their growth. Accordingly, in agreement with our findings the results of Stewart and Flinn (1984, on *Pinus eldarica*), Phillips *et al.* (1986, *Pinus eldarica*), Ostos *et al.* (2007, on *Pistacia lentiscus*) show that faster growth of tree occurs in the effluent-irrigated areas. This is mostly due to high nutrient concentration in effluent. It may be also noted that the nutrient contents in the municipal effluent is more than needed by plants whereas in the such conditions trees can produce greater biomass (Fitzpatrick *et al.*, 1986; Martinez *et al.*, 2003; Sing and Bhati, 2005; Guo *et al.*, 2006). Regarding the differences indicated above and positive effects of effluent on the growth of *P. eldarica*, it can be recommended that the produced huge municipal effluent in south of Tehran can be used for accomplishment of plantation projects and for development of rural and urban green spaces and green belts around the city and for reduction of air pollution, too. It is necessary to clarify that the decision for each location should be made based on accurate management, chemical, physical and microbial characteristics of water, soil and plant, according to international standards.

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Parasitological Contamination in Organic Composts Produced with Sewage Sludge

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1. Introduction

The activities performed daily by humans generate large volumes of waste from various areas, increasing environmental pollution and public health problems. According to the recommendations of the World Health Organization (WHO), the reactor effluent treatment from domestic sewages can be used in agriculture, since it applied to cultures that provide little risk of contamination with pathogens (Ayres & Mara, 1996). The sewage sludge is classified as class A when origins of processes with effective reduction of pathogens and can be used without restrictions in horticulture, and in the class B if results of processes of moderate reduction of pathogens, with more restricted use and be applied in reforestation and other cultures in which the risk of environmental and human contamination can be better controlled (Fernandes, 2000; David, 2002).

Sewage sludge must have characteristics that allow its setting within the parameters set for each class (David, 2002). For class A, the most probable number of coliforms per gram of dried sludge must be less than 1000, and the parasitic contamination should be less than one viable egg of helminths in four grams of dried sludge and less than one egg per litre of effluent (WHO, 1989; Fernandes, 2000).

The processes most often employed for the stabilization of sewage include the aerobic and anaerobic digestion. The application of lime and the composting are also recommended in some countries like USA, France and Brazil. However, the efficiency of the stabilization processes depends of the operational quality and of the pathogen characteristics present in the sewage sludge (Paulino et al., 2001).

2. Parasites presented in sewage sludge

Several countries have researched alternatives for final disposal of the waste from water sewage and sludge treatment. The sewage, prior to the stabilization treatment and disinfection, can contain macro and micronutrients and many pathogenic microorganisms and parasites. The handling and use of sewage and sludge obtained, without prior treatment, may promote severe infection to humans and animals (Paulino et al., 2001). According to WHO, 25% of the world's hospital beds are occupied by patients with diseases transmitted by contaminated water. About 1.5 billion people are infected with *Ascaris lumbricoides* and 1.3 billion with *Ancylostoma* spp. infection (Crompton, 1999).

The establishment of maximum concentrations of viable eggs of helminths in sewage sludge has been the criterion worldwide used to allow the agricultural use of this material (Capizzi-Banas & Schwartzbrod, 2001). Epidemiological studies have shown that the high frequency of helminths in human population, the long survival of the eggs in the environment and the low infectious dose are risks associated with agricultural use of sewage sludge (Soccol et al., 1997). In the world 3.5 billion people are infected with helminths or protozoa and children are the most frequently contaminated, resulting in approximately 60 000 deaths associated with *Ascaris lumbricoides* each year and 70 000 with *Entamoeba histolytica* (WHO, 2000). Table 1 showed the main pathogenic helminths eliminated in human faeces.

Helminths	diseases	cycle characteristics
Ancylostoma duodenale	ancylostomiasis	human – soil – human
Ascaris lumbricoides	ascaridiasis	human – soil – human
Necator americanus	necatorosis	human – soil – human
Trichuris trichiura	tricurosis	human – soil – human
Taenia saginata	taeniasis	human – cattle – human
Taenia solium	taeniasis	human – pigs – human
Taenia solium	cysticercosis	human – soil - human

Roque, (1997).

Table 1. Main pathogenic helminths from human faeces

3. Sewage sludge stabilization

The sludge stabilization is used primarily to reduce substantially the numbers of pathogenic organisms, to minimize health risks, controlling odours and to restrict the possibility of further decomposition. The stability is generally associated with the tendency to biodegrade organic matter. This step is an important in sewage treatment and influences significantly the characteristics of sludge produced (Fernandes, 2001).

The resulting sludge from biological treatment systems of wastewater is composed of live microorganisms. As the efficiency of biological processes is related to the amount of living cells, active in the process, the processing systems keeps the affluent rich in a medium with sludge. A biological process is considered efficient and economical when can be operated with low hydraulic retention times and long retention times of solids to allow the growth of microorganisms. The sludge is thus an initiating factor for the processes of biological treatment of sewage and its excess is regarded as a waste. The efficiency of stabilization and production of this waste depend of the technology of sewage treatment systems and its operationalization (Fernandes 2001).

Stabilisation processes can be divided on biologic and chemist process. Biological methods involve digestion anaerobic, aerobic digestion, autothermal aerobic digestion and composting. The alkaline is the most used chemical process and usually the lime is mixed with the material, improving pH that inactivates some pathogenic microorganisms. Other chemical agents can be chlorine ozone, hydrogen peroxide and potassium permanganate but to a small scale treatment.

4. Composting and parasitic contamination reduction

According to Metcalf & Eddy (1991), composting is an economically and environmentally correct alternative to the stabilization of wastes from agriculture and industries production and urban sewage treatment systems, with possible agronomic use of such waste. This technology involves the organic waste processing into humified composts through two distinct phases. The first corresponds to biochemical reactions of oxidation and the second to the process of humification or maturation phase. Being developed by a microbial ecosystem, the composting is directly influenced by factors that act on microbial activity. Among these, the most important are aeration, temperature, humidity content and nutrient concentration (Veras & Povinelli, 2004).

Large number of treatment plants in Brazil employs the natural composting process, in which organic material, after separation of insensitive material for composting, is arranged in stacks in the courts and are periodically raked to improve aeration. However, there are some cases using expedited procedures with injection of air into the piles (Barrier et al., 2006).

5. Parasitological contamination in compounds with sewage sludge before and after thermal treatment

The temperature is an important factor to contamination reduction in the composting. Study conducted in Brazil had as objective to evaluate the contamination by eggs, cysts and oocysts of protozoa in organic compounds, using sewage sludge and agricultural residues, before and after heat treatment (Duarte et al., 2008). In the experiment conducted in Montes Claros, Minas Gerais, Brazil, 25 different organic compounds were prepared, with approximate volume of 0.5 cubic meters. The materials were kept during the first fifteen days, and daily irrigated and raked (Duarte et al., 2008).

The compounds were prepared with carbon /nitrogen relation of 25:1, 30:1, 35:1, 40:1 and 45:1. In the composition were used cotton waste (5.8 to 10%), seeds of maize and beans (11.8 to 17.2%), and grass seeds (4.4 to 4.7 %). Were also used coconut fibber (50-20%), rice husk (7.4 to 17%), chopped elephant grass (*Pennisetum purpureum*) (1.93 to 17.6%), shell of *Cariocar brasiliense* (pequi) (0.6 to 2.27%), fresh cattle manure (2.3 to 4.8%) and sewage sludge (28.8 to 38.1%). Faeces were from 40 Holstein cows with an average of 5.5 years. Each compound received 220g of rock phosphate, 21kg of gypsum and 21kg of limestone (Duarte et al., 2008).

First two samples of 500g sewage sludge were collected from the Juramento Town, in North of Minas Gerais, Brazil. The sludge was collected in the dry bed of the sewage treatment station of this local that uses flow anaerobic reactor ascending. The physical and chemical analysis of sewage sludge were performed according to techniques recommended by Tedesco et al. (1995) and showed pH-H_20 4.8 and levels of nitrogen, carbon and humidity of 2.4, 9.4 and 6.0%, respectively (Duarte et al., 2008).

Subsequently, were collected 500g samples from compounds with fifteenth day of composting, avoiding contamination from one compound to another and put them in plastic bags, clean and free from contamination by parasites. After this period, the compounds were transferred to a greenhouse with a controlled temperature of 60°C during 12 hours and subsequently to keep 55°C until the twenty-second day after treatment. This procedure was employed with the purpose of promoting the reduction of pathogens parasites of the compounds. The effectiveness of heat treatment and their uniformity was checked in all the materials produced and three different samples from several parts of these compounds corresponding to the parts A, B and C, were collected after twenty-two days of thermal treatment. Was obtained 500g for each sample (A, B and C), using the same hygienic measures to prevent the contamination (Duarte et al., 2008).

5.1 Processing of samples and parasitological exams

Apart from two samples of sewage sludge was collected in all 100 samples of the produced compounds, with 25 before heat treatment and 75 after treatment (samples A, B and C). The samples were mixed one to one and 10 grams were weighed and stored with 30 ml of 10% formaldehyde solution for preservation of eggs, cysts and oocysts present (Duarte et al., 2008).

In the quantification of helminth eggs, protozoan cysts and oocysts, was used the technique of sedimentation of solids during 12 hours after the addition of filtered water to complete one litter. After this procedure, the method Bailenger modified by Ayres & Mara (1996), recommended by WHO was used. Two MacMaster chambers were prepared and for each sample were counted only viable eggs, obtaining the arithmetic mean of the two counts (Ayres & Mara, 1996).

Aiming to check the viability of eggs observed on microscopy was performed quantitative faecal culture technique described by Ueno, (1998). Two grams of the samples before heat treatment and after heat treatment were weighed (corresponding to B samples). The material was mixed with two grams of autoclaved dry sawdust and 10 ml of 1% sulfuric acid solution to fungi growth control. After 28 days of incubation at room temperature, was used the Baermann technique for collection and Keith (1953) key for the identification of larvae from samples obtained before and after heat treatment.

After counting of eggs and larvae of nematodes and protozoa cysts and oocysts from the 25 compounds analyzed, the data were transformed into log (X +1) and submitted to the Student's t-test at 5% probability for comparison of the contamination before and after heat treatment.

5.2 Parasitological contamination of sewage sludge

In two s sewage sludge sample from Juramento city, North of Minas Gerais State of Brazil, the results revealed 1.2×10^4 eggs, 10^4 cysts and oocysts/kg for first sample and 2.7×10^4 eggs and 2.7×10^4 cysts and oocysts/ kg of the second sample, respectively. No infective larvae were recovered after quantitative faecal culture, indicating that the process of anaerobic digestion of sewage may have compromised the development and hatching of nematode larvae (Duarte et al., 2008).

In regions with hot weather, the utilization of up flow anaerobic sludge blanket (UASB) is a positive option for the treatment of domestic sewage. However, workers handling in these processes and the produced wastes are potential risk of being infected by parasites (Carvalho et al., 2003; Souza et al., 2006). The effects of aerobic and anaerobic digestion during 15 days on nematode eggs were evaluated by Black et al. (1982). The results indicated that 23% of *Ascaris* spp. eggs were destroyed in the anaerobic treatment and 38% with the aerobic. However, the anaerobic process had no effect on *Trichuris* spp. eggs and none of these treatments was effective for the *Toxocara* spp. eggs.

In a research in the region of Curitiba city, Parana State of Brazil, the contamination of sewage sludge obtained in anaerobic fluidized sludge was evaluated. Were observed significant reductions in the number of viable eggs, present in the material from different

treatment stations, ranging from 59.7 to 93%. The effectiveness of egg reduction was influenced by time and temperature processes and for seasonal effects (Paulino et al., 2001).
In another study on the Paraiba State, Northeast of Brazil, was evaluated the effectiveness of the post-treatment. The three investigated systems were wetlands, rock beds and polishing ponds. The results indicated that the raw sewage and UASB effluents from the reactor had averages 353.7 and 50 eggs per litter, respectively. *Ascaris lumbricoides* prevailed on the other species in the raw sewage, with relative frequency of 56.5%. The three systems of post-treatment effluent produced wastes free of helminth eggs and this could be explained by sedimentation produced by a hydraulic surface of 0.20 m day-1 in ponds operated (Souza et al., 2005).

5.3 Parasitic contamination in the compounds before and after thermal treatment

In the table 2 are described the quantification of larvae recovered, counting helminth eggs and protozoan cysts and oocysts in organic compounds before and after heat treatment. These results demonstrate a high parasitic contamination of all the compounds analyzed and statistical analysis indicated no reduction of this contamination after heat treatment (Duarte et al., 2008). All samples were with levels above of one egg per 4 g of compost, which is not recommended for class A biosolids (Fernandes, 2000).
After the larval culture of the compound samples, before of the heat treatment, was observed that 52.4% of the larvae were of the genus *Cooperia*, 36.7% of the genus *Trichostrongylus*, 7.1% *Bunostomum* spp. and 3.8% to *Haemonchus* spp. After heat treatment of the compounds, 33.3% of the larvae were of the genus *Cooperia*, 30.7% of the *Trichostrongylus* spp., 2.7% to infective larvae of *Strongyloides* spp., which are commonly found as parasites of ruminants, and 33.3% of free living forms of the genus *Strongyloides* (Duarte et al., 2008).
The two samples of sewage sludge and the 100 samples of organic compounds showed high counts of viable helminth eggs under light microscopy. However, larvae of the cultures were recovered only in samples from the compounds and the genus identification suggested that the contamination came from the cattle manure or agricultural wastes contaminated with nematode eggs from ruminants (Duarte et al., 2008).

Compounds	eggs x 10^4/kg		larvaes x 10^4/kg		cysts e oocysts x 10^4/kg	
	before	after	before	after	before	after
avarage	33.9	22.3	25.3	41.9	52.8	66.6
standard deviation	31.3	21.2	23.6	71.5	54.7	82.3

(Duarte et al., 2008).

Table 2. Parasitic contamination in 25 different organic compounds produced with sewage sludge and agricultural residues before and after heat treatment at 60 ° C for 12 hours in North of Minas Gerais State of Brazil

Table 3 describes the average count of eggs, cysts and oocysts of protozoa from samples of three separate parcels (A, B and C) of each one of the 25 compounds, after the heat treatment at 60°C for 12 hours. There were no significant differences between samples of the same compound ($P < 0.05$). The results revealed high counts of these parasitic structures in both the samples of the compounds after heat treatment, indicating that this process was not effective in the reducing of parasitic contamination in any of the sampled points (Duarte et al., 2008).

The data indicate that, even after composting and thermal treatment, the parasite eggs can remain viable and produce infective larvae. The compounds produced in this study could be classified as class B, for use on crops with little risk of contamination in relation to pathogenic organisms, such as cotton, orchards and plantations. These compounds also should not be applied to pasture for ruminants, since could be the source of contamination for gastrointestinal nematodes (Duarte et al., 2008).

The use of compounds with sewage waste for the fertilization of pastures should also not be displayed, because it could constitute a risk for contamination of the cattle with *Taenia saginata* eggs, contributing to the permanence of both cycles of the bovine cysticercosis and human taeniasis (Duarte et al., 2008).

compound Parcels	eggs x 10^4/kg			cyts and oocysts x 10^4/kg		
	A	B	C	A	B	C
averege	15.2	22.3	11.0	38.2	66.6	35.8
standard deviation	16	21.2	11.5	51.4	82.3	31.7

Duarte et al. (2008)

Table 3. Contamination with helminth eggs, cysts and oocysts of protozoan in three different samples of organic compounds produced from sewage sludge and agricultural residues after heat treatment at 60 ° C for 12 hours in the North of Minas Gerais, Brazil

6. Different waste treatments and parasitological contamination

Gaspard et al. (1995) also showed that nematode eggs are strongly resistant to most of the classical waste treatments. The work was carried out on sludges from various origins to properly evaluate the impact of the different treatments on nematode eggs. An extraction followed by a concentration procedure allowed isolation of eggs, the viability study being then performed on a culture. For the 19 samples with live eggs, all types of treatment were represented: fresh sludge, prolonged aeration, anaerobic digestion, lagooning, composting and liming. No egg development inhibition phenomenon was observed in fresh sludge. The test demonstrated there were 93% viable eggs. Biological treatments do not produce a total inactivation of nematode eggs. The samples submitted to such various treatments as prolonged aeration, anaerobic digestion, lagooning or composting still showed higher percentages of viable eggs. Prolonged aeration seemed to be totally ineffective with 93%

viable eggs recovered, and a lagoon containing 6-year-old sediments still showed 26% viable eggs. Regarding anaerobic digestion, 66% of viable eggs were recovered in the one sample. For the compost, the analysis on a small number of 8 eggs showed a viability of 25% and the chemical treatment with lime after 20 days of storage gave 66% of viable eggs.

Jhonson et al. (1998) evaluated an in-vitro test for the viability of *Ascaris suum* eggs exposed to various sewage treatment processes. After one week in a mesophilic anaerobic digester, 95% of *A. suum* eggs produced two-cell larvae in vitro, with 86% progressing to motile larvae. After five weeks in the digester, 51% progressed to motile larvae. Between 42% and 49% of eggs stored in a sludge lagoon for 29 weeks were viable and able to develop motile larvae. In the case of eggs that were embryonated before treatment, > 98% survived up to five weeks in the digester and were able to develop motile larvae. More than 90% of embryonated eggs survived for 29 weeks in the sludge lagoon and were able to develop motile larvae.

Solid waste landfill leachate and sewage sludge samples were quantitatively tested for viable *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Encephalitozoon cuniculi* spores by the multiplexed fluorescence in situ hybridization (FISH) assay. Depending on the variations utilized in the ultrasound disintegration, sonication reduced the load of human-virulent microsporidian (obligate intracellular parasites) spores to no detectable levels in 19 out of 27 samples (70.4%). Quicklime stabilization was 100% effective, whereas microwave energy disintegration was 100% ineffective against the spores of *E. bieneusi* and *E. intestinalis*. Top-soil stabilization treatment gradually reduced the load of both pathogens, consistent with the serial dilution of sewage sludge with the soil substrate. This study demonstrated that sewage sludge and landfill leachate contained high numbers of viable human-virulent microsporidian spores and that sonication and quicklime stabilization were the most effective treatments for the sanitization of sewage sludge and solid waste landfill leachates (Graczyk et al., 2007).

Kouja et al. (2010) assessed the presence and loads of parasites in 20 samples of raw, treated wastewater and sludge collected from six wastewater treatment plants. Samples were tested by microscopy using the modified Bailenger method (MBM), immunomagnetic separation (IMS) followed by immunofluorescent assay microscopy, and PCR and sequence analysis for the protozoa *Cryptosporidium* spp. and *Giardia* spp. The seven samples of raw wastewater had a high diversity of helminth and protozoa contamination. *Giardia* spp., *Entamoeba histolytica/dispar*, *Entamoeba coli*, *Ascaris* spp., *Enterobius vermicularis,* and *Taenia saginata* were detected by MBM, and protozoan loads were greater than helminth loads. *Cryptosporidium* sp. and *Giardia* sp. were also detected by IMS microscopy and PCR. Six of the eight samples of treated wastewater had parasites: helminths (n=1), *Cryptosporidium* sp. (n=1), *Giardia* sp. (n=4), and *Entamoeba* (n=4). Four of five samples of sludge had microscopically detectable parasites, and all had both genus *Cryptosporidium* sp. and *Giardia* sp. and its genotypes and subtypes were of both human and animal origin.

In another study evaluated the process of anaerobic digestion for treatment of cattle manure. After larvae cultures, positive results were obtained for the L3 larvae of *Haemonchus* spp., *Oesophagostomum* spp. and *Cooperia* spp. in the effluent, even after forty days of retention time (Amaral et al., 2004). However, Padilla & Furlong (1996) observed inactivating effect of anaerobiosis, close to 100%, with the retention time above of 56 days and according to Olson & Nansen (1987), mesophilic anaerobic digestion (35° C) and thermophilic (53° C) accelerated the inactivation of nematodes in relation to survival time of these parasites in conventional storage.

Sewage sludge and slurry are used as fertilizers on pastures grazed by ruminants. The interest of application on pastures of these two biowastes is environmental (optimal recycling of biowastes) and agronomic (fertilisation). The parasitic risk and the fertilisation value of such applications on pastures were evaluated during one grazing season. The sludge group of calves did not acquire live cysticerci and thus the risk was nil under the conditions of the study (delay of 6 weeks between application and grazing). The slurry group of calves did become lightly infected with digestive-tract nematodes, mostly *Ostertagia ostertagi*. Under the conditions of this experiment, a 6-week delay between application and grazing strongly reduced the risk of infection (Moussavou-boussougou et al., 2005a).

Helminth infection acquired by lambs grazing on pastures fertilised either by urban sewage sludge or cattle slurry were studied by Moussavou-boussougou et al. (2005b) in temperate Central Western France. The aim was to assess the risk of larval cestodoses in lambs after sewage application and of digestive tract nematode infection following the slurry application. The lambs did not acquire cysticercosis or any other larval cestodoses in the sewage sludge group and only very limited infections with *Cooperia* spp. and *Nematodirus* spp. were observed in the slurry group. It was concluded that the helminth risk was extremely low and was not a cause of restriction of the use of these biowastes.

7. Conclusion

The results obtained in the North of Minas Gerais, Brazil, showed that even after the composting of agricultural waste with sewage sludge and heat treatment at 60°C for 12 hours, large numbers of helminth eggs can remain viable. The use of the compounds with sewage sludge should be allocated to perennial crops and low risk of contamination for animals and humans is therefore not recommended for grazing ruminants, for horticulture or for the production of edible mushrooms.

The variation in data of other research to reduce parasitic contamination in composting and anaerobic digestion processes indicates the need for further research, standardizing and monitoring the waste to be recycled for agricultural or other purposes, to reduce risks to public health and animal infection. The initial contamination of sewage sludge used as well as time and temperature of the composting should be elucidated and the final compost produced should always be monitored as to risk of parasitic contamination that could be present.

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Effects of Reclaimed Water on Citrus Growth and Productivity

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1. Introduction

Sewage wastewater or effluent is often viewed as a disposal problem. However, it can be a source of water for irrigation, creating an alternative disposal method for wastewater treatment facilities, benefiting agriculture as an alternate source of irrigation water, and reducing the demand for use of surface or ground water for irrigation (Parsons et al., 2001a and b). Treated wastewater, also known as reclaimed water, is typically treated municipal sewage from which excess plant nutrients, organic compounds and pathogens have been removed. The terms wastewater, treated wastewater and reclaimed water will be used interchangeably in this chapter.

The characteristics and treatment of these treated waters will be described and discussed in this chapter along with use as an irrigation source for citrus production. Potential disadvantages of using reclaimed water for agricultural irrigation include real or perceived concerns about reductions in surface and ground water quality (i.e. nutrients and heavy metals), harmful effects on workers that come in contact with treated wastewater (i.e. organic compounds and pathogens), and the safety of crops for human consumption (i.e. carcinogens and pathogens) (Parsons & Wheaton, 1996; Parsons et al., 1995). In some arid regions where freshwater supplies are limited, irrigation with reclaimed water is already commonly practices (Feigin et al., 1991). Israel was a pioneer in the development of wastewater re-use practices, but was quickly followed by many other countries (Angelakis et al., 1999). Israel and the Palestinian Autonomous Regions, for example, are projected to use 3500 million m^3 of water in 2010, with 1400 million m^3 (40% total water supply) used for irrigation. Treated sewage water used for irrigation would be approximately 1000 million m^3 or 70% of agricultural water demand and will play a dominant role in sustaining agricultural development (Haruvy, 1994). Wastewater is a preferred marginal water source, since its supply is reliable and uniform, and is increasing in volume due to population growth and increased awareness of environmental quality (Haruvy & Sadan, 1994). Costs of this water source are low compared with those of other unconventional irrigation water sources (e.g. desalinization) since agricultural reuse of urban wastewater serves also to dispose of treated urban sewage water (Haruvy & Sadan, 1994). Total cost of supplying wastewater for agricultural reuse (i.e. treatment, storage and conveyance costs) minus total costs of alternative safe disposal (e.g. deep well injection and wetlands creation) must be considered when developing wastewater reuse systems (Angelakis et al., 1999; Arora&Volutchkov, 1994; Haruvy, 1997)).

2. Wastewater reuse: the general case

The rapid development of irrigation has resulted in an increased water demand. Accessible water resources (e.g. rivers and shallow aquifers) in most agricultural areas are now almost entirely committed (Angelakis et al., 1999). Alternative water resources are therefore needed to satisfy further increases in demand. This is particularly a necessity in regions which are characterized by severe mismatches between water supply and demand. Low water resource availability and temporal symmetries in availability result in water provided for human consumption and other urban use with less water for agricultural use. The reduction in water availability for agriculture can lead to reduced sustainability of agricultural enterprises and/or environmental problems (Angelakis et al., 1999). One potential alternate source of irrigation water for agriculture situated near large urban centers is treated wastewater. Reclaimed water contains many nutrients essential for plant growth, and may have an effect similar to that of frequent fertigation with a dilute concentration of plant nutrients (Neilsen et al., 1989). In addition, recycling these nutrients may prevent pollution of surface or ground water (Sanderson, 1986).

In the Mediterranean basin, wastewater has been used as a source of irrigation water for centuries. In addition to providing a low cost water source, the use of treated wastewater for irrigation in agriculture combines three advantages 1) using the fertilizing properties of the water can partially eliminate synthetic fertilizers demand and contribute to decrease nutrient concentration of rivers, 2) the practice increases the available agricultural water resources, and 3) in some areas, it may eliminate the need for expensive tertiary treatment (Angelakis et al., 1999).

In a review by Haruvy (1997) wastewater quality or treatment levels are defined by various constituents such as 1) organic matter- biochemical oxygen demand, chemical oxygen demand and total suspended solids; 2) organic pollutants (i.e. stable organic matter that may affect health); 3) trace elements resulting from industrial water use; 4) pathogenic microorganisms; 5) potential plant nutrients (e.g. N and P); and 6) salinity. Treatment processes are generally divided into primary, secondary and advanced or tertiary processes. Primary treatment includes basic treatment such as screening to remove coarse solids and solid precipitates. Secondary treatment includes low-rate processes (e.g. stabilization or sediment ponds) with high land and low capital and energy inputs, and high rate processes (e.g. activated sludge) with low land and high capital and energy inputs (Pettygrove & Asano, 1985). Tertiary stages of treatment further improve water quality by nitrification and denitrification to reduce water N.

Environmental hazards may be caused by each constituents (e.g. nutrients, heavy metals) left in wastewater and may leach below the root zone increasing groundwater pollution (Feigin et.al, 1990). Salinity of reclaimed water is generally within acceptable ranges and often lower than other irrigation sources, however, salinity levels may be acceptable only for ground application and not direct plant contact in some treatments processes (Basiouny, 1982). Leaching of fertilizers, pesticides and salts from soils irrigated with treated wastewater or over application of poor quality wastewater has resulted in the progressive loss of subsurface water quality and decrease in groundwater resources in some areas (Lapena et al., 1995). However, when properly managed, the use of treated wastewater in agriculture to conserve water resources and to safely and economically dispose of wastewater is a very feasible option.

Treated municipal wastewater has become an important potential source of irrigation and plant nutrients and has been used successfully in the production of high yield marketable quality crops for decades (Allen & McWhorter, 1970; Crites, 1975; Day, 1958; Henry et al., 1954; Stokes et al., 1930). The response of plants and soils to municipal treated effluent is dependent on the quality of the applied effluent and nature and efficiency of the wastewater treatment, with generally higher treated water resulting in the best growth and yields (Basiouny, 1984). Recently, wastewater has been used to increase yield and improve quality of grain crops (Al-Jaloud et al., 1993; Day & Tucker, 1977; Day et al., 1975; Karlen et al., 1976; Morvedt & Giovdane, 1975; Nguy, 1974), cotton (Bielorai et al., 1984; Feigin et al., 1984), forage (Bole & Bell, 1978) and vegetable crops (Basiouny, 1984; Kirkham, 1986; Neilsen et al., 1989 a, b, c, 1991; Ramos et al., 1989). Reclaimed water has been successfully used to irrigate many fruit crops; apples (Nielsen et al., 1989a), cherries (Neilsen et al., 1991), grapes (Neilsen et al., 1989a), peaches (Basiouny, 1984) and citrus (Esteller et al., 1994; Kale & Bal, 1987; Koo & Zekri, 1989; Morgan et al., 2008; Omran et al., 1988; Wheaton & Parsons, 1993; Zekri & Koo, 1990).

3. Guidelines for wastewater reuse in irrigation

The Ganga is the most important river system in India. It rises from the Gangotri glacier in the Himalaya mountains at an elevation of 7138 m above mean sea level as a pristine river. Half a billion people (almost one tenth of the world's population) live within the Ganga river basin at a average density of over 500 per km^2 (Singh et al., 2003). This population is projected to increase to over one billion people by 2030. Sewage treatment plants provide agricultural benefits by supplying irrigation and nonconventional fertilizers in the Ganga river basin as an alternate disposal of effluent into the river (Singh et al., 2003). Areas with extensive agriculture and rapidly escalating population must use water resources in a sustainable way and require guidelines to insure the health of the population and maintain water quality and the environment in sensitive areas such as the Gana river basin.

Wastewater reuse guidelines typically cover four areas for each application (i.e. type of crop irrigated): chemical standards, microbiological standards, wastewater treatment processes and irrigation techniques (Angelakis et al., 1999). The degree of treatment required and the extent of monitoring necessary depend on the specific use and crop. In general, irrigation systems are categorized according to the potential degree of human exposure.

The highest degree of treatment is always required for irrigation of crops that are consumed uncooked (Angelakis et al., 1999). However, wastewater is often associated with environmental and health risks. As a consequence, its acceptability to replace other water resources for irrigation is highly dependent on whether the health risks and environmental impact entailed are acceptable. Evaluation of reusing wastewater is the quality of the water in terms of the presence of potentially toxic substances or of the accumulation of pollutants in soil and crops. It is important to access the source of the wastewater for heavy metals from industries or synthetic chemicals normally present in urban wastewater (e.g. oils, disinfectants). There have also been debates about applicable microbiological practices and the type of crops that should be irrigated with treated effluent (Asano & Levine, 1996). One set of guidelines established in California, USA and now accepted nationwide and other countries of the world, promote very high water quality standards (comparable to drinking water standards), confident that costly treatment practices provide safe enough water (i.e. free of enteric viruses and parasites) for who can afford it. The "California criteria" (State of California, 1978) stipulate conventional biological wastewater treatment followed by tertiary treatment, filtration and chlorine disinfection to produce effluent that is suitable for irrigation (Arora & Voutchkov, 1994). In support of this approach, Asano & Levine (1996) reported two major epidemiological studies conducted in California during the 1970s and 1980s. These studies scientifically demonstrated that food crops irrigated with municipal wastewater reclaimed according to the California approach could be consumed uncooked without adverse health effects. However, the nutrients removed by the tertiary treatment are not available for fertilizing of the crops.

In contrast to the California approach, the guidelines produced by the World Health Organization (WHO) are less stringent and require a lower level of water treatment (WHO, 1989). The WHO guidelines are, however, more restrictive in assuring microbiological quality of treated water, requiring monitoring of fecal Coliform bacteria (also required in the California criteria) as well as human parasitic nematodes.

Outside of Europe, other countries (e.g. Israel, South Africa, Japan and Australia) have chosen criteria more or less similar to those adopted by California (and elsewhere in the US). Most countries in Europe accept the 1989 HWO guidelines but contain additional criteria such as treatment requirements and/or use limitations in order to ensure proper public health protection. The California approach has the most data in its own support and thus has been accepted by more countries because of its "safety first" philosophy but is the most expensive to implement.

4. Risk assessment

Shuval et al. (1997) developed a preliminary model for the assessment of risk of infection and disease associated with wastewater irrigation of vegetables eaten uncooked based on a modification of the Haas et al. (1993) risk assessment model for drinking water. The modifications included determining the amount of wastewater that would cling to irrigated vegetables and estimates of the concentration of pathogens that would be ingested by consuming vegetables irrigated with wastewater of different propagule densities. The model was validation with data from a cholera epidemic caused in part by consumption of wastewater irrigated vegetables and provided reasonable approximation of the levels of disease that really occurred. The risk assessment, using this model, of irrigation with treated wastewater effluent meeting the WHO guidelines (WHO, 1989, 1,000 fecal coliform bacteria 100 ml^{-1}) indicates the risk of contracting a virus disease is about 10^{-6} to 10^{-7}. Regli et al. (1995) concluded that guidelines for drinking water standards should be designed to ensure that human populations are not subjected to the risk of infection by enteric disease at > 10^{-4} for a yearly exposure. Thus this preliminary study suggested that the WHO guidelines provided a factor of safety some 1 to 2 orders of magnitude greater than that called for by the United States Enviornmental Protection Agency (USEPA) (USEPA, 1992) for microbial standards for drinking water.

5. Wastewater irrigation of Florida citrus: a case study

Florida has experienced rapid growth in population during the last 50 years with a 5.5-fold population increase from 1950 to 2000 (U.S. Census Bureau, 1997; Perry & Mackum, 2001;

Smith, 2005). Groundwater withdrawal for domestic and irrigation use has increased by 15.5 and 20.7 times, respectively, during the same period (Marella & Berndt, 2005). Likewise, the amount of wastewater generated by cities in Florida has increased more than five-fold since 1950. Environmental concerns about degradation of surface waters by treated effluent water have caused many communities to consider advanced secondary treated wastewater (reclaimed water) reuse. Currently there are 440 reclaimed water reuse systems in Florida irrigating 92,345 ha with 2,385 million liters of reclaimed water per day (Florida Department of Environmental Protection, 2005). The majority of these systems irrigate golf courses, public right-of-ways, and home landscapes. However, 6,144 ha of production agriculture are currently irrigated with reclaimed water, with citrus (*Citrus* spp L.) orchards accounting for all but 364 ha (Morgan et al., 2008).

Florida citrus production benefits from irrigation because the average annual rainfall of more than 1200 mm is unevenly distributed throughout the year with approximately 75% of annual rainfall occurring from June to September (Koo, 1963). Furthermore, Florida citrus trees are grown on sandy soils with very low water holding capacity, particularly orchards in the central "ridge" portion of the state. Typical available water content values for central Florida ridge citrus soils range from 0.05 to 0.08 cm^3 cm^{-3} (Obreza & Collins, 2003). Increased water use by the growing population and localized water shortages during low rainfall years have resulted in the development of water use restrictions, and decreases in permitted water use for agriculture. Increased use of reclaimed water for agricultural irrigation would not only reduce the wastewater disposal problem for urban areas, but could also reduce the amount of water withdrawn from surficial and Floridan aquifers for irrigation.

Water for irrigation is no longer abundant and restrictions on the use of available groundwater in agriculture are becoming more severe. Due to the increasing demand for water, water use for agricultural purposes has become strictly regulated in Florida (Koo & Zekri, 1989; Wheaton & Parsons, 1993; Zekri & Koo, 1990). Additionally, urban growth, especially in the coastal areas of Florida, has increased the need for efficient and environmentally safe disposal of reclaimed water. The Department of Environmental Regulation (FDER) has restricted the disposal of municipal reclaimed water into lakes, rivers and streams, so alternative disposal sites need to be found (Maurer & Davies, 1993).

Wastewater has been recognized as a possible important source of major plant nutrients (e.g. N, P and K), although the chemical composition of wastewater varies between locations (Berry et al., 1980). Long term studies using reclaimed water to irrigate citrus for up to 60 years in Egypt found no adverse effects on tree growth compared to ground water irrigated citrus (Omran et al., 1988). Similarly, irrigation with reclaimed water increased growth and yield of citrus on well drained sandy soils of the Florida Ridge with no adverse affects on health and yield of mature trees (Koo & Zekri, 1989; Zekri & Koo. 1990). Similar results were observed for young citrus trees grown of well drained soils (Wheaton & Parsons, 1993).

Soil types and drainage patterns of the poorly drained flatwoods soils near the Florida coastline vary considerably due to the presence of a high water table (Maurer & Davies, 1993). The potential waterlogging of the fatwoods hold problems not associated with citrus grown on the Ridge. In a three year study, trees grown of poorly drained sandy soils were irrigated with a simulated reclaimed water, simulated reclaimed water with fertilizer added or ground water with fertilizer added for a period of three years after planting (Maurer & Davies, 1993). Trees irrigated with simulated reclaimed water and ground water with fertilizer added had significantly larger canopies and trunk diameters than trees irrigated with simulated reclaimed water only indicating that use of reclaimed water alone was insufficient to support adequate growth of young citrus trees.

Prior to 1987, the City of Orlando and Orange County wastewater treatment plants discharged their effluent into Shingle Creek, a tributary of Lake Tohopekaliga (Zekri & Koo, 1989). Faced with the need to expand wastewater treatment volume and a state requirement to eliminate discharge of treated effluent to surface waters, the city and county entered a negotiated settlement with the Florida Department of Environmental Regulation and the United States Environmental Protection Agency to cease effluent discharge into Shingle Creek and develop an innovative reclamation program (Zekri & Koo, 1989). Initial funding of $180,000,000 established the project which is called Water Conserv II (Parsons et al., 2001a). The Water Conserv II/Southwest Orange County Water Reclamation Project (Conserv II) involves the use of highly treated wastewater for citrus irrigation and groundwater recharge. It is one of the largest water reuse projects in the United States and the first reuse program permitted in Florida that involves irrigation of crops intended for human consumption. The program, which became fully operational in January, 1987, currently delivers approximately 133,000 cubic meters of reclaimed water per day (cmd) (275,000 cmd maximum flow) to approximately 1750 ha of citrus. Other users of reclaimed water from the Water Conserv II project are eight foliage greenhouse operations, four tree farms, two ferneries, and three golf courses. The reclaimed water is distributed though 80 km of pipelines maintained by the project. Excess reclaimed water is disposed of in 71 ha of rapid infiltration basins that recharge surficial and Floridan aquifers. Water Conserv II is the largest reclaimed water agricultural irrigation project of its type in the world and was the first project in Florida to be permitted to irrigate crops for human consumption with reclaimed water (McMahon et al., 1989).

Citrus groves in western Orange and eastern Lake Counties, Florida (lat. 28° 28′ 20″ N, long. 81° 38′ 50″ W, elevation 64 m) were selected for the Conserv II project because of their high demand for irrigation water and soil series which have high permeability. The predominant soil order in this area is Entisol, with Candler fine sand (hyperthermic, uncoated, Typic Quartzipsamment) being the dominant soil series (Obreza & Collins, 2003). The Candler series consists of excessively drained, very rapidly permeable soils formed from marine deposits. These soils are located in upland areas and typically have slopes of 0-12%. The A and E horizons consist of single-grained fine sand, have a loose texture, and are strongly acidic (pH = 4.0 – 5.5). A Bt horizon is located at a soil depth of 2 m and includes loamy lamellae of 0.1 to 3.5 cm thick and 5 to 15 cm long. This area is a primary aquifer recharge area for the lower Florida peninsula (Zekri & Koo, 1989). Use of reclaimed water for irrigation, in lieu of previous surface water discharges, benefited the urban sector by 1) reducing competition from the agricultural demand for potable water and 2) increasing available groundwater supplies through supplementing natural recharge of the aquifer. The agricultural sector benefited from the project by 1) providing citrus growers with a long-term source of water that will increase and not decrease with urban growth and 2) reduced irrigation pumping costs associated with deep wells previously used for irrigation.

To receive reclaimed water for irrigation at no cost, citrus growers were required to sign a contract with the City of Orlando and Orange County to accept 1270 mm of water per year for a period of at least 20 years. Initially, there was grower resistance because of concerns that use of the reclaimed water might damage citrus trees, or make the fruit unmarketable. As part of the contract, the growers requested long-term studies on the effects of reclaimed water on citrus tree health and fruit quality. Orchards were not now required to accept the full 1270 mm of water per year under the contract because rapid infiltration basins (RIBs) were installed in the early 1990s. Due to the highly porous nature of the soils, the RIBs function as alternate disposal sites (particularly during the normally wet summer rainy season) where the reclaimed water is applied at high rates and allowed to percolate to the ground water. Still questions persisted regarding the effect of long-term use of wastewater on tree productivity.

Conserv II water is good quality water having low mineral concentrations and very low TDS (Zekri & Koo, 1990). Characteristics and chemical composition of reclaimed water provided by the Water Conserv II project are summarized in Table 1. This treated wastewater is highly treated having relatively low biological oxygen demand and mineral contents. In general, growers in the project have followed sound irrigation practices (Koo & Zekri, 1989; Zekri & Koo, 1990). An initial survey of orchards receiving reclaimed water from Conserv II was conducted from 1986 to 1989. No adverse affects of reclaimed water use on tree health and productivity were noted in the initial phase of the orchard survey, however, continued monitoring was suggested to determine long term effects (i.e. metal accumulation in soil, leaves or fruit).

Leaf samples indicated that both trees irrigated with reclaimed wastewater and ground water were adequately fertilized. No consistent trends were observed for leaf K, Ca, Mg and Cu contents. Although leaf Na content from trees irrigated with reclaimed wastewater was twice as high as trees on well water, Na content of both groups was well within the optimum standard values for citrus (Obreza & Morgan, 2008). While the surface six inches of soil did not show any consistent trends due to irrigation with reclaimed water, accumulation of nutrient elements became more apparent when the soil profile to one meter was examined. Higher N and P were found in the soil profile of reclaimed water irrigated groves in 1988 when compared to the well water groves. No differences were observed in the extractable soil K, Ca, Mg and Na of reclaimed water and control groves. Fruit from trees irrigated with reclaimed water had lower soluble solids and acid content in 1987 than fruit from control trees. Such effects of irrigation on juice quality are well documented (Koo & McCornack, 1965; Koo & Smajstral, 1984). In 1987, soil water content was considerably higher in the reclaimed water groves than the control groves resulting in lower soluble solids. In 1988, soil water content in the reclaimed groves was only slightly higher than the control groves and differences in soluble solids were not detected.

A long-term replicated small plot study was conducted from 1989 to 2000 to determine the affect of irrigation with reclaimed water on citrus trees on sandy soils and irrigated with water supplied by the Water Conserv II project (Parsons et al., 2001b). Reclaimed water was applied to citrus trees from planting to 10 years of age at 400, 1500 and 2500 mm per year at equal monthly amounts. Ground water applied at recommended rates based on daily evapotranspiration was provided as a control. The highest two treated wastewater irrigation rates promoted greater trunk and canopy growth. In the first three years, trunk diameters were similar for the ground water control and 400 mm rate of reclaimed water. From years four to 10, trees that received the 1250 and 2500 mm per year application rates were significantly larger than those receiving the 400 mm treatment. The 2500 mm per year reclaimed water rate produced well, even though the high irrigation rate caused a significant reduction in juice soluble solids, 19.3% more fruit per hectare than the 400 mm per year treatment resulting in 15.5% total soluble solids per hectare compared with the 400 mm rate because of the greater fruit production at the higher irrigation rate. These results show that irrigation with excessively high rate of reclaimed water was not detrimental to canopy growth and fruit production. This was due to the good drainage of this sandy soil and the lack of root diseases. The slight reduction in juice soluble solids at the high irrigation rate was more than compensated for by the higher total soluble solids yield.

In the same study, leaf N contents were slightly lower in plants irrigated with groundwater than wastewater (Parsons et al., 2001b). It was concluded that this was due to elevated levels of organic matter found in wastewater which provided additional N. Higher leaf N was also found in treated wastewater irrigated sweet-cherry (Neilsen et al.,1991), apples (Neilsen et al., 1989c), cotton (Feigin et al., 1984) and peach trees (Basiouny, 1984). No significant differences in leaf P contents were found between plants irrigated with either groundwater or wastewater, in spite of wastewater supplying a higher soil P load. This is explainable considering that the amount of P supplied by both kinds of irrigation water was a small percentage of total P from soil and fertilizer sources. Leaf K, concentration in leaves of plants irrigated with groundwater was significantly higher than in plants irrigated with wastewater probably because the elevated Na levels in the wastewater inhibited K uptake by citrus plants (Banuls et al., 1990). Soil solution Na has been found to antagonize K uptake in other plants (Epstein, 1961; LaHaye & Epstein, 1969; Cramer et al., 1987). Plants irrigated with wastewater showed higher leaf content of Cl and Na than those irrigated with groundwater. Citrus is considered to be a salt sensitive crop (Mass & Hoffman, 1977) and salinity causes reduction in growth, ionic imbalance, and adverse water relations in citrus (Walker et al., 1982). Embleton et al. (1973) established 0.7% and 0.25% as the limit for Cl and Na concentrations, respectively. Above these tissue concentration limits, toxic effects are manifested in citrus. No salinity effects were observed over the 10 year study because the nearly 950 mm rainfall during Florida's rainy season (June to Septhermber) does not allow for accumulation of salts.

A second orchard monitoring project to determine any adverse effects on citrus tree health and production associated with long-term irrigation using reclaimed water started in 1995 and was terminated in 2004 (Morgan et al., 2008). The objective of this project was to determine whether long-term irrigation with treated municipal wastewater 1) reduced tree health (i.e. canopy appearance and leaf nutrient content), 2) decreased visual fruit loads, 3) impacted internal fruit quality (i.e. Brix, titratable acid, Brix:acid ratio, and/or 4) increased in soil contaminant concentrations. In 1994, 10 orchards irrigated with one of the two water sources were selected for a total of 20 orchards. These 20 orchards were paired so that trees of the same scion and relative age were irrigated with either water sources. The scions used were 'Hamlin' and 'Valencia' oranges (*C sinensis* L.), 'Sunburst' tangerine (*C. reticulata* Blanco), and 'Orlando' tangelo (*C. reticulata* Blanco x *C. paradisi* Macfadyn) however, the root stocks were not always consistent among the two water sources. Random trees over a four hectare plot in each orchard were evaluated quarterly for canopy appearance, leaf color, fruit crop, and weed cover. Each orchard received a separate visual rating for each category on a 1-5 scale. A rating of 1 indicates a less dense canopy compared with visual inspection of orchards in the area at the same time period, leaf color would be chlorotic and/or have visual deficiency symptoms, the fruit crop would be low enough to be unharvestable, and the weed population would be very low indicating insufficient nutrition, soil water content or excess herbicide application. Ratings of 5 would indicate a thick dense canopy with excessive vegetative growth, dark green leaves with N concentrations above that considered optimum, a fruit crop considered to be well above the average for trees of comparable age and size in the area, and a dense weed population in the herbicide zone well in excess of standard grower practices.. Fruit samples (20 fruit) were taken from five trees in each orchard just prior to harvest and analyzed for percent juice content, Brix, acid, and weight. Degrees Brix and total titratable acidity were determined according to methods approved for Florida citrus quality tests (Wardowski et al., 1995).

Samples of spring growth leaves (20 leaves from five trees) and soil (two cores from each of five trees were taken from each orchard in Aug. or Sept. of each year from 1994 to 2004. Leaf samples were analyzed for N, P, K, Ca, Mg, Na, Zn, Mn, Fe, and B. Soil samples were taken at the same time to a depth of 60 cm and were analyzed for P, K, Ca, Mg, Zn, Mn, Al, Cu, Fe, Na, and Cl.

Citrus orchards in this project were irrigated with either groundwater or reclaimed water. Orchards irrigated with groundwater were managed using recommended practices receiving 30 to 40 cm of irrigation per year. However, orchards irrigated with reclaimed water had higher soil water content (Zekri & Koo, 1993), presumably because of more frequent irrigation. Orchards irrigated with reclaimed water had soil moisture content of 0.06 cm^3 cm^{-3} compared with 0.05 cm^3 cm^{-3} for orchards irrigated with ground water. Field capacity was estimated to be 0.65 cm^3 cm^{-3} for these soils, indicating that orchards irrigated with reclaimed water were near or above field capacity a higher proportion of the time compared with orchards irrigated with ground water. The quality of the reclaimed water used for irrigation was monitored monthly, and a report of average water constituent concentrations was provided to the growers (Table 1). The level of constituent concentrations in the reclaimed water are not considered to be toxic (Burton & Hook, 1979; Feigin et al., 1984). However, if soil or tissue accumulation were to occur, concentrations of heavy metals (i.e. cadmium, lead, and zinc) may approach toxic levels (Campbell et al., 1983; Feigin et al., 1984; Neilsen et al., 1991).

Prior to 1994, Zekri & Koo (1993) reported that soil to a depth of 0.5 m beneath trees irrigated with reclaimed water was usually 14.7 mm higher in water content and the trees had 6% higher canopy, leaf color, and fruit crop ratings than trees irrigated with groundwater. The higher ratings were attributed to consistently higher soil water content in the orchards irrigated with reclaimed water. For the period 1994 to 2004, mean quarterly canopy appearance, leaf color, and fruit crop, were significantly higher in orchards irrigated with reclaimed water compared with orchards irrigated with groundwater. Weed growth in orchards irrigated with reclaimed water was consistently higher, but not significantly different, than orchards irrigated with well water. The difference in mean rating for the four categories was 12.3% possibly indicating greater water use in reclaimed water blocks compared with orchards irrigated with well water.

Mean canopy, leaf color, and fruit crop ratings for trees irrigated with ground water were significantly greater than ratings from 2000 to 2004 compared with trees irrigated with the same water source from 1996 to 1999. Whereas, canopy, leaf color, and fruit crop ratings for the orchards irrigated with reclaimed water did not have a similar pattern. Reduced canopy appearance, leaf color, and fruit set in orchards irrigated with groundwater can be attributed to reduced rainfall from 1994-1999 (390 mm, 1998) compared with average rainfall from 2000 to 2004 (1191 mm). Significantly lower tree appearance in a drought year agrees with conclusions of Zekri & Koo (1993) that commercial citrus orchards irrigated with reclaimed water were commonly irrigated more frequently and/or with a greater volume than those irrigated with groundwater.

Weed growth as measured by weed cover ratings was higher in reclaimed water irrigated orchards for most years compared with those irrigated with groundwater. Higher weed growth ratings have been correlated with high irrigation rates of reclaimed water (Parsons

	Drinking water MACL	Well water typical concentrations[1]	Conserv II reclaimed water MACL	Typical Conserv II reclaimed water concentrations[1]
	-------------------------------------	mg L^{-1}	------------------------------	
Arsenic	0.05	--	0.10	<0.005
Barium	2	--	1	<0.01
Beryllium	0.004	--	0.10	<0.003
Bicarbonate	--	--	200	105
Boron	--	0.02	1.0	<0.25
Cadmium	0.005	--	0.01	<0.002
Calcium	--	39	200	42
Chloride	250	15	100	75-81
Chromium	0.1	--	0.01	<0.005
Copper	1	0.03	0.20	<0.05
EC (µmhos)	781	360	1100	720
Iron	0.3	0.02	5	<0.4
Lead	0.015	--	0.1	<0.003
Magnesium	--	16	25	8.5
Manganese	0.05	0.01	0.20	<0.04
Mercury	0.002	--	0.01	<0.0002
Nickel	0.1	--	0.20	0.01
Nitrate-N	10	3	10	6.1-7.0
pH	6.5-8.5	7.8	6.5-8.4	7.1-7.2
Phosphorus	--	0.01	10	1.1
Potassium	--	6	30	11.5
Selenium	0.05	--	0.02	<0.002
Silver	0.1	--	0.05	<0.003
Sodium	160	18	70	50-70
Sulfate	250	23	100	29-55
Zinc	5	0.02	1	<0.06

[1] As reported in Parsons et al., 2001b.

Table 1. Maximum allowable contaminate limit (MACL) for Florida drinking water and Conserv II reclaimed water, and typical Water Conserv II reclaimed water concentrations. All values are in mg L^{-1} except for pH and EC.

& Wheaton, 1992; Zekri & Koo, 1993). As with tree appearance and fruit crop, weed cover ratings only were significantly lower for orchards irrigated with groundwater in 1998 compared with other years, presumably due to lower rainfall. Growers have adjusted their herbicide practices to reduce the negative impact of increased weed growth due to higher irrigation use with reclaimed water by reducing reclaimed water use or increasing herbicide applications.

In five out of 11 years (1994, 1995, 1998, 2000, and 2001), mean fruit juice content or the percent of fruit weight in juice were significantly higher among trees in orchards irrigated with reclaimed water rather than ground water. These years with significant juice content differences among irrigation water sources lead to a significant year by water source interaction for Juice content. Juice soluble solids or Brix was not significantly different among water sources. However, Brix were significantly different among water sources in 1994, 1997 and 1998 contributing to a significant year by water source interaction. Two of these years were considered dry years with below normal rainfall. Fruit weight were significantly higher for orchards irrigated with reclaimed water compared with fruit from orchards irrigated with ground water, however, no year * water source interaction was noted. Therefore, higher fruit crop ratings, fruit weights, and similar solids per fruit (during normal rainfall years) in orchards irrigated with reclaimed water would suggest similar or greater yields in terms of soluble solids per ha compared with orchards irrigated with groundwater. The previous study by Koo & Zekri (1989) found that reduced soluble solids and acid concentration in the juice was correlated with higher soil water content in the orchards receiving reclaimed water. Likewise, significant differences in fruit Brix and acid were seen in this study from 1994 to 1998, but not after 1998. This change in fruit Brix and acid my indicated a change in irrigation practices with orchards being irrigated with similar amounts some time after 1998. This shift in irrigation practice would correspond with construction of RIBs and reduced requirement for the use of reclaimed water. Because fruit yield was greater from orchards irrigated with reclaimed water, total soluble solids produced per ha were higher in the reclaimed water orchards than the groundwater irrigated orchards.

Irrigation with reclaimed water has increased soil concentrations of P, K, Mg, B, Na, and Cl when reclaimed water was used as an irrigation water source (Burton & Hook, 1979; Campbell et al., 1983; Feigin et al., 1984; Neilson et al., 1991). Elemental concentrations in soil samples taken in Aug. or Sept. of each year from orchards irrigated with either reclaimed or ground water varied from year to year but were not significant by years. Calcium was the only element significantly different by soil sample depth with higher concentrations found near the surface. This result was expected since calcium applied as lime applied for pH adjustments in orchards irrigated with either groundwater or reclaimed water, and Ca in the reclaimed water would be incorporated into this layer with little leaching over time. With the exception of increased P, Ca and Al no elements were found to be significantly different when comparing water sources. Soil in orchards irrigated with reclaimed water was significantly higher for P, Ca and Al compared with soils in orchards irrigated with ground water. However, no elements were found to be excessive (Maurer & Davies, 1993; Tucker et al., 1995). Lower extractable soil K was found in orchards receiving higher rates of reclaimed water despite the higher K concentration of reclaimed water. These data are consistent with findings of Zekri & Koo (1993) who reported P, Ca, and Mg were significantly higher and K significantly lower in soil samples from orchards irrigated with reclaimed water compared with orchards irrigated with groundwater.

Calcium was the only element with years * water source and depth * water source interactions. Soil calcium concentrations were significantly lower (1034.7 kg ha^{-1}) in years with normal rainfall (2000-2004) compared with dryer years (1338.5, 1996-1999). Differences in soil Ca concentration among the two irrigation water sources followed the same pattern during these years with soil from orchards irrigated with reclaimed water have higher concentrations than did soil from orchards with ground water (data not shown). Likewise, soil Ca concentrations followed the same pattern with depth regardless of irrigation water source resulting in higher concentrations in soil irrigated with reclaimed water at the selected depths compared with soil from orchards irrigated with ground water.

Leaf sample elemental concentrations were generally higher from orchards irrigated with reclaimed water compared with orchards irrigated with groundwater. While higher, significantly higher P and Ca concentrations in soils irrigated with reclaimed water did not lead to significantly higher leaf concentrations. These results can be explained by dilution of leaf concentration by increased biomass production of trees irrigated with reclaimed water, reduced nutrient uptake efficiency, or a combination of the two. Unfortunately, differences in biomass accumulation were not determined in this study. However, only Mg and B were significantly higher in leaf samples from orchards irrigated with reclaimed water compared with samples from orchards irrigated with groundwater. Zekri & Koo (1993) found significantly higher Fe and B concentrations in more than half the years between 1987 and 1993. Based on this information, it is now recommended that orchards irrigated with reclaimed water not add B to micronutrients sprays. Zekri & Koo (1993) found significantly higher Na and Cl concentrations in leaf samples from orchards irrigated with reclaimed water, presumably from higher irrigation applications. However, Na and Cl were not significantly different from 1994 to 2004, further indicating a change in irrigation practice among orchards irrigated with reclaimed water.

6. Conclusion

Few detrimental effects on citrus orchards have been associated with irrigation using the reclaimed water. However, the impact of using reclaimed water on groundwater contamination have not been determined. Appearance of trees irrigated with reclaimed water was usually better, with higher canopy, leaf color, and fruit crop ratings, than orchards irrigated with groundwater. Higher weed growth in reclaimed water irrigated orchards was associated with higher soil water content. However, growers apparently have made adequate adjustments to their herbicide practices. Higher soil water content in the orchards receiving reclaimed water resulted in reduced fruit soluble solids. However, because fruit crop ratings and larger fruit size indicated greater fruit yield, total soluble solids produced per ha were similar to or higher in the reclaimed water irrigated orchards than in the groundwater irrigated orchards. Irrigation with reclaimed water generally increases soil P and Ca, and reduces soil K. Reduction of P and Ca and increases in K applied to citrus orchards irrigated with reclaimed water may be required adjustments in fertilizer applications to citrus orchards irrigated with reclaimed water. Likewise, leaf B concentration increased in most citrus trees irrigated with treated wastewater, requiring an adjustment in foliar nutrient application practices.

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18

Heavy Metal Contamination of Zn, Cu, Ni and Pb in Soil and Leaf of *Robinia pseudoacacia* Irrigated with Municipal Wastewater in Iran

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1. Introduction

The economic development of the society towards large-scale urbanization and industrialization is leading to production of huge quantities of wastewaters (Singh & Agrawal, 2008). Wastewaters can be used for the restoration of degraded land (Madejo´n et al., 2006), and the growth of vegetation having commercial and environmental value (Aggeli et al., 2009). Establishment of tree plantations following wastewater irrigation has been a common practice for many years (Kalavrouziotis & Arslan-Alaton, 2008). Several researches of wastewater irrigated plantations in many countries such as India (Bhati & Singh, 2003; Singh & Bhati, 2005), Australia (Sharma & Ashwath, 2006), New Zealand (Guo et al., 2002; Kimberley et al., 2003), Sweden (Hasselgren, 2000), Canada (Cogliastro et al., 2001), Hungary (Vermes, 2002), etc. are available.

In Iran, huge section of useful water of major metropolitan cities converts to the municipal wastewater (Tajrishi, 1998). Since the deficiency of access to adequate water for irrigation is a matter of increasing concern and limiting factor to develop plantation, therefore municipal wastewater could be utilized as an important source of water for expansion of tree plantations in and around the city and industrial complexes (Al-Jamal et al., 2000; Kalavrouziotis & Apostolopoulos, 2007; Salehi et al., 2007). This practice not only reduces the toxicity of soil and plays an important role in safeguarding the environment, because woody species may utilize wastewater and uptake heavy metals through extensive root systems and retain them for a long time (Madejo´n et al., 2006), but it also creates opportunities for commercial biomass production and sequestration of excess minerals in the plant system (Sharma & Ashwath, 2006).

Again, wastewaters may contain amounts of potentially harmful components such as heavy metals and pathogens (Rattan et al., 2005; Toze, 2006). The effects of microbial pathogens are usually short term and vary in severity depending on the potential for human, animal or environmental contact (Toze, 2006), while the heavy metals have longer term impacts that could be a source of contamination and be toxic to the soil (Sharma et al., 2007) and plant (Gasco´ & Lobo, 2007). Hence if wastewater is to be recycled safely for irrigation, the problems associated with using it need to be known (Emongor & Ramolemana, 2004).

According to differences in climatic, vegetation, socio-economic conditions and also in quality of soil and wastewater between different regions and even within different time periods in one region, utilizing only the applicable guidelines to other regions of the world would be a mistake and in long-term would damage the soil and water resources, therefore local researches need to be carried out (Kalavrouziotis & Arslan-Alaton, 2008).

Robinia pseudoacacia L. (black locust) is native to the southeastern United States, but has been widely planted and naturalized elsewhere. *R. pseudoacacia* trees have nitrogen- fixing bacteria on its root system, for this reason it can grow on poor soils, therefore it can improve fertility of soil. In Iran it often planted alongside streets, in green space and parks, especially in large cities, because it tolerates pollution well (Mossadegh, 1993). The use of municipal wastewater in growing *R. pseudoacacia* in suburban areas could be beneficial for the economic disposal of wastewater, defers ecological degradation by containing the pollutants in the soil and growth of vegetation having aesthetic and environmental value. The present study was carried out around Tehran, Iran, where wastewater has been commonly used for irrigation of peri-urban crops for many years. The objective of the present report is to quantify concentration and contamination of Zn, Cu, Ni and Pb in irrigation water, soil and leaves of *R. pseudoacacia* trees from site having long-term use of wastewater for irrigation of land.

2. Materials and methods

2.1 Site description

The study site is located in Shahr-e Rey, 5 Km south of Tehran-Iran (Latitude 35° 37' N, Longitude 51° 23' E, 1005 m above sea level). The climate of the area is semi-arid with mild-cold winters and 7 months (Mid April-Mid November) dry season. Average annual rainfall and average annual temperature are 232 mm and 13.3° C, respectively. The highest rainfall is in March (41.32 mm) and the lowest in August (0.89 mm). The warmest month occurs in August and the coldest in January.

Experiments were conducted at two even-aged (15 years) artificial stands of black locust in October 2006. The first stand was irrigated with municipal wastewater and the second with well water since they were planted. Durations of irrigation were based on tree water-use and the potential evapo-transpiration, which varied seasonally in response to the climate and on an average the irrigations were carried on 8 day durations for 8 months/year (April-November). The soil of two stands were both clay-loam (according to US soil taxonomy) with 29.25% clay, 36.20% silt and 34.55% sand in the stand irrigated with municipal wastewater and 27.14% clay, 37.86% silt and 35% sand with well water (Table 2).

2.2 Plant and soil sampling

For the sampling of leaf and soil, four plots were randomly identified in each stand. Plots were 30 m × 30 m, with tree spacing of 3 m × 4 m. In each plot, four trees were selected and in the growing season leaf samples of *Robinia pseudoacacia* trees taken from the top of crown and the part affected by sunlight (Habibi Kaseb, 1992). This collection provided 16 leaf samples in each stand. At the end of the sampling, one representative leaf sample from each plot (by mixing of four samples of each plot) was taken (decreasing of sample quantity for chemical analysis). Soil samples were taken under each selected tree from the root zone at a depth interval of 15 cm down to 60 cm by digging profiles. This collection provided 48 soil samples in each stand from three depths (0-15, 15-30 and 30-60 cm). At the end of soil sampling, three representative soil samples of three depths from each plot were taken by mixing of samples of each layer in each plot (decreasing of sample quantity for chemical analysis) according to Habibi Kaseb (1992). Municipal wastewater and well water were sampled daily (3 days in each month) from early June to late November at three-hour intervals (7 am, 13 pm and 19 pm) to make a composite sample of each day.

2.3 Laboratory analysis

Concentrated HNO_3 was added to the water samples to avoid microbial utilization of heavy metals (Sharma et al., 2007) and then they were brought to the laboratory in resistant plastic bottles to avoid adherence to the container wall. They were filtered through a Whatmann 42 mm filter paper and stored at 4 °C to minimize microbial decomposition of solids (Yadav et al., 2002; Bhati & Singh, 2003). Some parameters were measured separately, pH and EC by the procedure described using OMA (1990) and heavy metals (Zn, Cu, Ni and Pb) of water samples were estimated by the aqua regia method of Jackson (1973) followed by a measurement of concentrations using an Atomic Absorption Spectrophotometer (model-3110, Perkin-Elmer, Boesch, Huenenberg, Switzerland).

The soil samples air-dried, crushed, passed through a 2 mm sieve and were analyzed for various physico-chemical properties. Soil texture was determined using the hydrometer method according to Bouyoucos (1965). Soil pH and electrical conductivity (EC) were determined in 1:2 soil:water suspension by pH and EC meters (Hati et al., 2007). Soil organic carbon (SOC) content was determined by the Walkley–Black method (Nelson & Sommers, 1996). Calcium carbonate ($CaCO_3$) was measured with a calcimeter. The concentration of soil heavy metals (Zn, Cu, Ni and Pb) was extracted after digestion with 3:1 concentrated HCl–HNO_3 and measured by Atomic Absorption Spectrophotometer (Gasco´ & Lobo, 2007).

Leaf samples were washed using tap water, rinsed with distilled water, oven dried at 80 °C for 24 h, ground in a stainless steel mill and retained for chemical analysis (Singh & Bhati, 2005). For determination of heavy metal concentration (Zn, Cu, Ni and Pb), the leaf samples were wet digested as per Jackson (1973) and were measured using an Atomic Absorption Spectrophotometer.

2.4 Statistical analysis

Average leaf heavy metals and soil physico-chemical properties of two stands (irrigated with municipal wastewater and irrigated with well water), compared using independent-samples t-test (Pelosi & Sandifer, 2003). Data of soil heavy metals were analyzed for differences due to depth in the profile using one-way ANOVA. Furthermore, the variations in EC, pH and heavy metals of municipal wastewater and well water were also tested using independent-samples t-test. All the data were analyzed using the SPSS statistical package (Lindaman, 1992).

3. Results and discussion

3.1 Physico-chemical properties of wastewater and well water

The quality of municipal wastewater and well water was assessed for irrigation with respect to their pH, EC, and concentration of heavy metals (Table 1). Results indicated that the waters were alkaline in reaction. The pH of the municipal wastewater in various months ranged from 7.51 to 7.75 and 6.69 to 7.62 for well water. The EC of wastewater ranged from 1.78 to 2.12 dS/m with the greatest value detected in August. The average EC of municipal wastewater exceeded 1 dS/m (1.91 dS/m) indicating that this wastewater was saline in nature (Rattan et al., 2005). The pH and EC of municipal wastewater were significantly ($P < 0.01$) higher than the well water. The concentration of heavy metals (Zn, Cu, Pb and Ni) tended to be higher in municipal wastewater. In water samples, Zn, Cu, Pb and Ni concentrations were 0.43, 0.09, 0.033 and 0.028 mg/l, respectively in well water samples, whereas, corresponding values for wastewater were 3.30, 1.26, 0.106 and 0.081 mg/l. On an average, wastewater contained 7.67, 14, 3.21 and 2.89 times higher amounts of Zn, Cu, Pb and Ni respectively compared to well water. The comparison of measured factors with WHO (World Health Organization) standard showed that water used for irrigation based on pH and EC were in a normal range, however based on heavy metals: Pb and Ni concentration of municipal wastewater and well water was higher than standard range. Zn concentration of municipal wastewater also was higher than the standard but Cu concentration was normal. The concentration of these two elements was lower than the standard in well water (Table 1).

Parameters	Units	Municipal wastewater	Well water	WHO*
pH	---------	7.63 ± 0.01^{a}	7.32 ± 0.05^{b}	6.5 - 8.5
EC	(dS/m)	1.91 ± 0.02^{a}	0.590 ± 0.008^{b}	3
Zn	(mg/l)	3.30 ± 0.06^{a}	0.43 ± 0.07^{b}	3
Cu	(mg/l)	1.26 ± 0.03^{a}	0.09 ± 0.01^{b}	1-2
Pb	(mg/l)	0.106 ± 0.063^{a}	0.033 ± 0.026^{b}	0.01
Ni	(mg/l)	0.081 ± 0.007^{a}	0.028 ± 0.002^{b}	0.02
Different superscripts in row indicate significant ($P < 0.01$) difference. Values are mean of eighteen replications (3 days * 6 months) with ± SE, * Hach, 2002				

Table 1. Characteristics of municipal wastewater and well water

3.2 Impact of municipal wastewater irrigation on soil properties

Data of Table 2 indicate that application of municipal wastewater were resulted an increase (0-60 cm soil layer; mean of soil layers) in pH, EC, C, organic matter and $CaCO_3$ of wastewater-irrigated soil as compared to well water-irrigated soil. Increase in pH was 1.02 unit and EC 1.68 times in soil of wastewater treatment compared to the soil of well water treatment. The increase in pH and EC of soil in the wastewater-irrigated stand may have been due to alkaline nature of municipal wastewater (Singh & Bhati, 2005). SOC as a basic index of soil playing a variety of roles in nutrient, water, and biological cycles (Rattan et al., 2005) was 1.17%–1.29% in municipal wastewater-irrigated soil, whereas it was 0.88%–1.14%

Soil properties	Clay (%)	Silt (%)	Sand (%)	texture	pH	EC (dS/m)	C (%)	Organic matter (%)	$CaCO_3$ (%)
Wastewater irrigated soil	29.25	36.20	34.55	Clay loam	8.17^{a} (0.03)	1.28^{a} (0.04)	0.718^{a} (0.032)	1.23^{a} (0.05)	20.20^{a} (0.57)
Well water irrigated soil	27.14	37.86	35	Clay loam	7.94^{b} (0.10)	0.763^{b} (0.036)	0.585^{b} (0.062)	1.00^{b} (0.107)	18.55^{b} (0.45)
Values are mean of four replications with ± SD in parentheses; Different superscripts in columns indicate significant ($P < 0.01$) difference									

Table 2. Soil properties of two stands (0-60 cm)

in soil irrigated with well water. Increase in SOC content might be due to municipal wastewater application (Bhati & Singh, 2003). In general, the suitability of soils for receiving wastewater without deterioration varies widely, depending on their infiltration capacity, permeability, cation exchange capacities, phosphorus adsorption capacity, texture, structure, and type of clay mineral (Ivan & Earl, 1972).

The concentration of heavy metals (Zn, Cu, Pb and Ni) was higher in all depths of wastewater irrigated soil compared to those of well water irrigated soil (Fig. 1). As a matter of fact, high concentration of heavy metals in wastewater leads to increase them in soil (Huerta et al., 2002; Nan et al., 2002; Mapanda, et al., 2005). The comparison of soil Zn, Cu, Pb and Ni with critical range of heavy metals in soil (Table 3) showed that only Ni of soil treated with municipal wastewater and Pb of soil treated with the both municipal wastewater and well water were higher than the standard amounts of soil. The effects of wastewater irrigation on accumulation of soil heavy metals depend on various factors such as concentration of wastewater heavy metals, the period of wastewater irrigation, and soil properties (pH, texture, organic matter) (Rattan et al., 2005). And also generally, 10 to 50 years is needed so that the heavy metal levels precede the standard levels (Smith et al., 1996). Because of the high concentration of Pb in all soil and water samples, it can be predicted that besides the municipal wastewater, Pb probably has been added to the water and soil from other sources such as air pollution.

In the present investigation the concentration of heavy metals decreased with soil depth in both stands (Fig. 2). These results are in agreement with the findings obtained later (Yadav

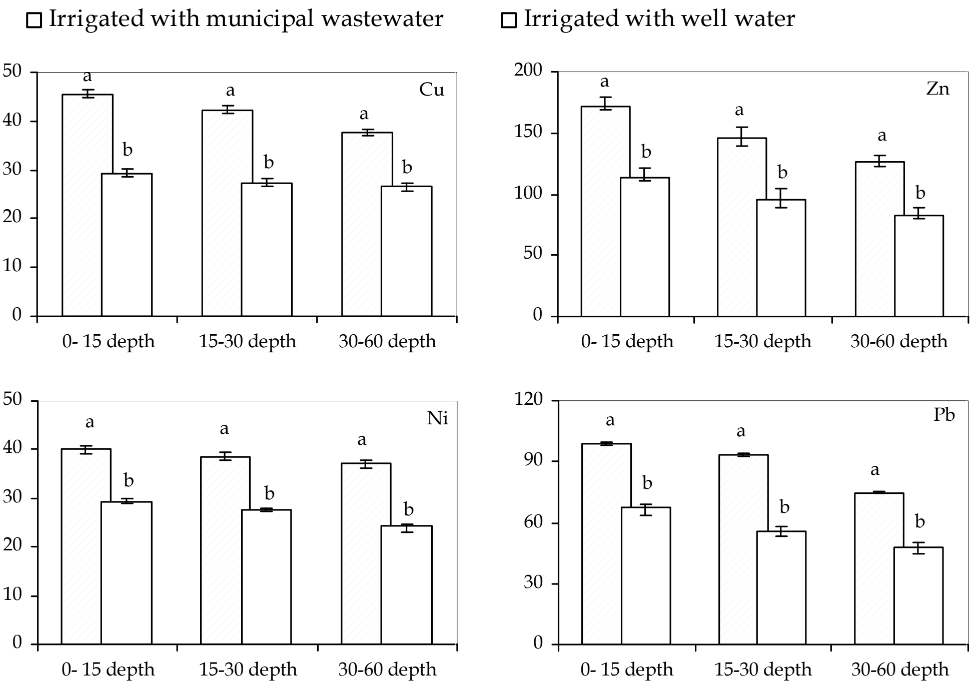

Fig. 1. Comparison of heavy metals in similar depths (0-15, 15-30 and 30-60 cm) between soils irrigated with wastewater and well water (mg/kg); Error bars are ± SE

et al., 2002). Since, the soil surface is richer in heavy metals than the underlying layers, greater accumulation in the topsoil probably is due to soil texture (the soil texture in both stands is clay-loam, as a result penetrability is decreased and accumulation of heavy metals are often observed at upper layers), low mobility of heavy metals in soil (Afyoni et al., 1998), and surface application of municipal wastewater.

Heavy metals	Critical range *
Zn (mg/kg)	10-500
Cu (mg/kg)	5-400
Pb (mg/kg)	40
Ni (mg/kg)	30

* Zn and Cu: Salardiny (1992); Pb and Ni: (EPA)

Table 3. Critical range of heavy metals in soil

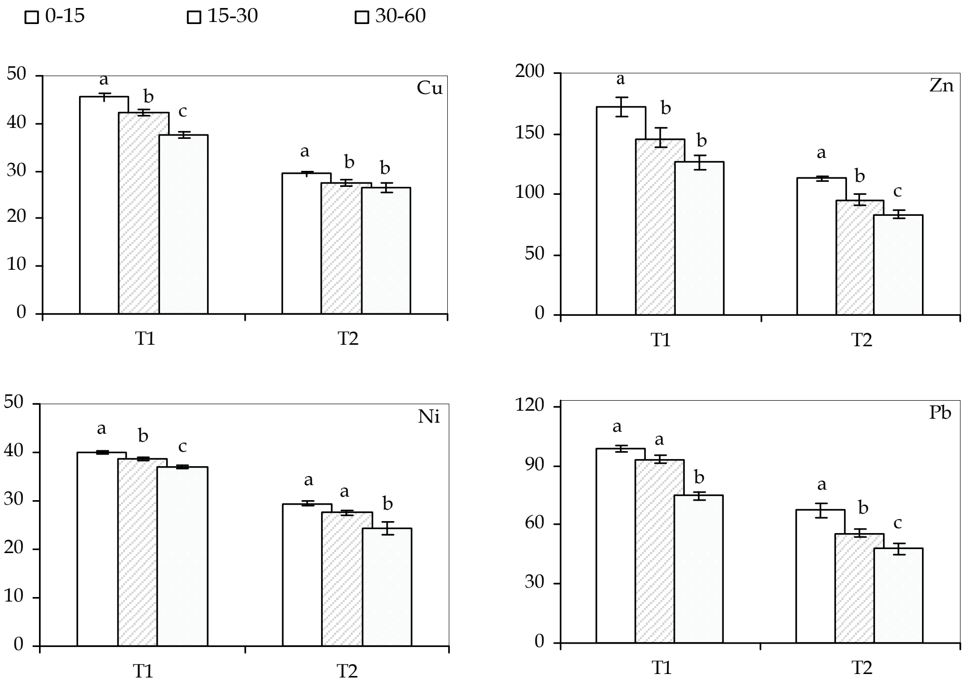

Fig. 2. Comparison of heavy metals among different depths (0-15, 15-30 and 30-60 cm) of soil in each irrigated stand (mg/kg); T_1: Soil irrigated with wastewater, T_2: Soil irrigated with well water; Error bars are ± SE

3.3 Changes in concentration of leaf heavy metals

The concentration of Zn and Cu elements in the leaves of black locust trees differed significantly under impact of two irrigation treatments. These concentrations in the leaves of wastewater irrigated trees were about 1.5 times higher than those of well water irrigation. However, irrigation with municipal wastewater did not result in toxicity to Zn and Cu of leaves (Table 4). Marked difference in Zn and Cu of tree leaves may be due to the increase of them through municipal wastewater (Meli et al., 2002). This result is in agreement with Singh & Bhati (2005) and Aghabarati et al. (2008), where substantially greater concentration of these elements were observed in leaf of *Dalbergia sissoo* seedlings and *O. europaea* trees irrigated with municipal wastewater compared to control. Ni and Pb were not detected in leaf samples which may be due to the low dynamic of heavy toxic metals, whereas it was likely accumulated in lower parts of the plant, such as root and stem. Nevertheless, Madejo´on et al. (2006) reported the presence of some heavy toxic metals in leaf of olive and holm oak trees. In fact, the quantity of element absorption using plant depends upon many factors including the total quantity of the elements applied through wastewater application, soil properties, and type of plant (Bozkurt & Yarilga, 2003; Kalavrouziotis and Arslan-Alaton, 2008).

Heavy metals	Wastewater	Well water	*P*-value	Range*
Zn (mg/kg)	30.62 ± 6.00 ^{a}	20.63 ± 2.60 ^{b}	<0.05	10-100
Cu (mg/kg)	4.87 ± 0.77 ^{a}	2.81 ± 0.23 ^{b}	<0.01	2-20
Ni (mg/kg)	nd	nd	------	-------
Pb (mg/kg)	nd	nd	------	-------
Values are mean of four replications with ± SD; different superscripts in rows indicate significant difference; nd: not detected; * Salardiny (1992)				

Table 4. Concentration of heavy metals in leaf of black locust trees irrigated with wastewater and well water

4. Conclusion

Today, the reuse of municipal wastewater for land irrigation constitutes a practical method of disposal which is expected to contribute decisively to the handling and minimization of environmental problems arising from the disposal of wastewater effluents on land and into aquatic systems. The application of wastewaters onto appropriate forest species will enable long term environmental protection, creating a new water source in significant quantities for the irrigation of forested areas at the same time. Again, the use of wastewaters for irrigating maybe increases heavy metals and pathogens in soil and plant. Hence, the control of all of parameters associated with the disposal of wastewaters on land should be done for safe reuse of them. Furthermore, the method and extent of use of wastewaters, however, vary according to the infrastructure and the local socio-economic conditions prevalent from country to country.

According to the results of the present paper from the area under study where municipal wastewater is being used for about 15 years, high level of some heavy metals in irrigation water and soil treated with municipal wastewater and possibility of accumulation of heavy toxic metals in lower parts of the plant, it is said that regulations about the utilization of municipal wastewater in irrigation should consider in order to minimize the risk of negative effects to ecosystem health. This can be controlled by avoiding toxic elements from entering the municipal wastewater and continued monitoring or treatment of wastewaters before it is put into disposal channel for irrigation.

5. Acknowledgments

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19

Wastewater from Table Olive Industries

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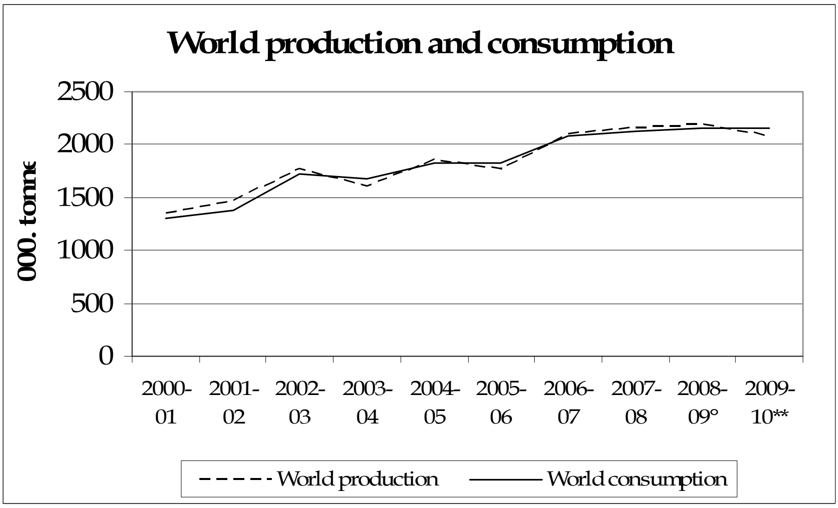
1. Introduction

In several Mediterranean countries the production of table olives plays an important part in the national economy. Moreover, in recent years there has been a worldwide increase in the production and consumption of these olives (Figure 1). From 2003 to 2009 the major olive-producing countries were: Spain (with an average of 503,300 tonnes per annum, representing approximately 26% of world production), Egypt (with an average of 299,600 tonnes per annum – 15.4% of world production) and Turkey (with an average of 230,800 tonnes per annum – 11.9% of world production). The major olive-consuming countries were: the USA; with an average of 217,600 tonnes per annum – 11.2% of world consumption), Spain, with an average of 197,700 tonnes per annum – 10.1% of world consumption) and Turkey (with an average of 183,700 tonnes per annum – 9.4% of world consumption).



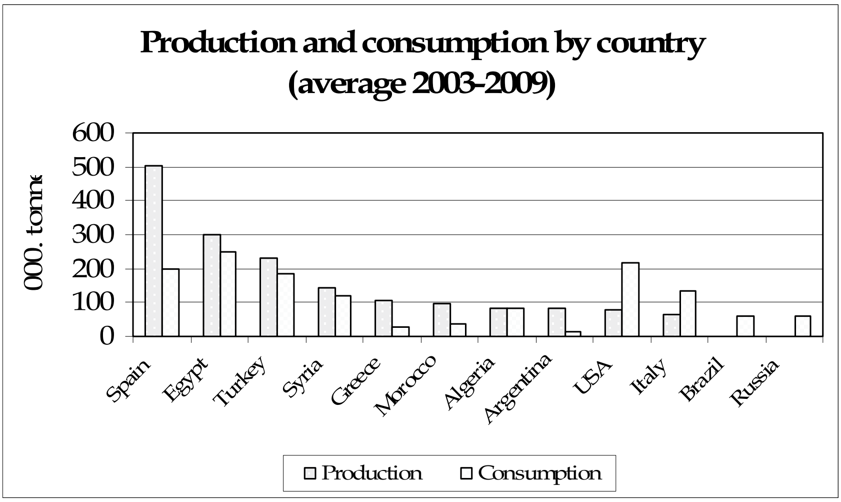
Source: www.internationaloliveoil.org

° provisional data **expected data

Fig. 1. Table olives world production and consumption

During the same period the major olive-exporting countries were: Spain (exporting an average of 181,700 tonnes per annum), Argentina (exporting an average of 65,000 tonnes per annum), Morocco (exporting an average of 63,500 tonnes per annum) and Egypt (exporting an average of 62,800 tonnes per annum). The countries which imported the most olives were: the USA (which imported an average of 129,600 tonnes per annum), Brazil (which imported an average of 61,400 tonnes per annum) and Russia (which imported an average of 55,800 tonnes per annum). (www.internationaloliveoil.org).

This chapter analyze the environmental aspects of table olives sector. After describing the production processes will be analyzed the characteristics of wastewaters, the pollution prevention technologies, and will be evaluate the relative environmental burdens through the LCA methodology.



Source: www.internationaloliveoil.org

Fig. 2. Table olives world production and consumption, by distinguishing among producers countries

2. The transformation of table olives

The quality of olives differs from year to year and depends on various things such as climate, rainfall, the amount of pests etc. The methods used for processing olives, according to the IOOC/Codex standard, are listed in Table 1. The degree of ripeness of the drupes when they are picked varies according to the processing method that is going to be used, as does the cultivar – which in some cases has taken on the name of the main area in which the olive-variety is used.

2.1 Green olives

Drupes which are harvested before they are completely ripe, i.e. when they are still green, are intended for processing as "Spanish-style green olives", "Castelvetrano style green olives" or "Naturally-processed black olives".

2.1.1 Spanish-style green olives

The most common method for producing green olives in brine is "Spanish-style" processing. This comprises the following steps: lye treatment (debittering), rinsing, brining, fermentation in brine, packaging and pasteurization.

GREEN OLIVES		TURNING COLOUR OLIVES		BLACK OLIVES		
Spanish-style	***Castelvetrano***	***Naturally***	***Naturally (Greek style)***	***Kalamata***	***Backed olives***	***Californian style***
Harvesting, sorting	Harvesting, sorting	Harvesting, sorting	Harvesting, sorting	Harvesting, sorting	Harvesting, sorting	Harvesting, sorting
Size sorting	Size sorting	Size sorting	Size sorting	Size sorting	Size sorting	Size sorting + (preservation in brine)
Lye treatment	Immersion in lye/salt solution	(Incision, cruscing, stoning)		Incision	Dripping	Lye treatment
Washing				Washing		Washing
In brine		In brine	In brine	In brine	Addition of dry salt	Immersion in ferrous salt solution
Fermentation	Fermentation	Fermentation	Fermentation	Immersion in vinegar	Drying oven	(Oxidation to air)
(Size sorting)		Size sorting	Size sorting (+ oxidation to air)			Size sorting
Packaging		Packaging	Packaging	Packaging + extravirgin olive oil	Packaging	Packaging
Pasteurization		Pasteurization				Sterilization

Table 1. Main methods of processing of table olives

Lye treatment (Debittering).

The olives are picked when they have reached their maximum size and are green, or greenish–yellow, in colour; then - after removing any leaves, sorting the drupes and grading them according to size - the olives are treated with an alkaline lye before being immersed in brine. During this treatment the olives are put into a dilute aqueous solution of sodium hydroxide, with a concentration of 1.7% - 4% (w/v); the strength of the concentration depends on the olive-variety, the degree of ripeness of the drupe and the temperature and characteristics of the water used. During the preparation of the lye, the reaction that occurs is exothermic, so the olives should not be put into the solution until it has cooled down. The strength of the concentration of sodium hydroxide depends on the characteristics of the olives being processed: for example, less ripe olives with harder flesh require a higher concentration of soda.

More concentrated solutions can soften the flesh of the drupe, while more dilute solutions - which slow down the debittering process - adversely affect the subsequent fermentation. During treatment the olives must be kept submerged in the solution to prevent oxidation by exposure to the air (resulting in blackening) and to avoid incomplete debittering. In addition, in order to stop the soda from collecting at the bottom of the container (which would result in a solution with varying degrees of alkalinity and thus a non-uniform softening of the drupes), the solution should be mixed and homogenized from time to time (Brighigna, 1998). This debittering phase of the process removes the oleuropein, one of the bitter glucosides naturally present in olive-flesh (Marsilio et al., 2001). The duration of the debittering process ranges from a minimum of 8 hours to a maximum of 15 hours; the treatment is considered complete when the NaOH solution has penetrated from 2/3 to 3/4 of the way into the olive-flesh. Traditionally olives have been treated using fresh lye, but it is possible to re-utilize exhausted lye and thus lower pollution levels (Garrido-Fernandez, 1997; Segovia-Bravo et al., 2008).

Rinsing.

The reason for rinsing the olives with water is to remove most of the lye from the flesh. This phase is very significant from an environmental point of view because it entails the use of large quantities of fresh water, with the consequent production of the same amounts of waste water which contains polluting compounds (Brenes, 2005).

The rinses may be:

- of long-duration: for olives which are meant to be consumed within a short period of time, or for those which are processed as semi-fermented olives; this method involves changing the rinsing water every 8-10 hours for a total duration of 4-5 days in order to obtain an almost clear liquid;
- of medium duration: this is the most commonly-used method. After a first rather short rinse lasting 1-2 hours there are then another 2 rinses lasting 8-12 hours each, resulting in a total of 18-25 hours of rinsing-time. In this case the olives retain enough fermentable substances to ensure proper lactic fermentation;
- of short duration: this consists of a single rinse lasting about 15 hours. With this method the large amount of NaOH solution left in the fruit prevents the rapid onset of lactic fermentation, and sometimes encourages abnormal fermentation. To ensure proper fermentation it is necessary to replace the used brine with fresh, resulting in the consumption of large quantities of fresh water. The elimination of the "brine mother",

however, results in the loss of important components such as fermentable substances, minerals, etc.;

- neutralizing rinses: the aim of this phase is to neutralize the alkalinity of the olives using organic acids (citric acid, ascorbic acid, acetic acid) or inorganic (hydrochloric) acid, or by means of the insufflation of micronized CO_2 into the brine. This procedure reduces water consumption and shortens processing time, while conserving most of the fermentable substances and encouraging a rapid onset of lactic fermentation. Some researchers have looked into the possibility of replacing the traditional rinses with a process which neutralizes the residual alkali using organic or inorganic acids (Brighigna, 1998; Garrido-Fernandez, 1997; Higinio Sánchez Gómez, 2006).

Fermentation in brine.

After rinsing or neutralization, the olives are placed in suitable containers and covered with brine that has a NaCl concentration starting at 9-10% but decreasing rapidly to around 5%, because of the high water content of the olives. Fermentation helps to preserve the product and improve its taste.

It is also possible to add used brines which have been previously analyzed; these are the so-called "brine mothers" which ensure the onset of safe lactic fermentation.

The expression 'onset of fermentation' means the moment when the brine has arrived spontaneously at a pH value of around neutral (± 7), while the fermentation process is deemed complete when, after 2-3 months, the olives are pale yellow in colour and have good texture and a pleasantly-sour taste. If more than 6 months lapses between the stage of fermentation and that of packaging, it is necessary to add salt to the solution in order to stabilize the brine at a concentration ranging from 8% to 10% (Arroyo-López, 2008; Brenes, 2004; Gomez et al., 2006; Garrido-Fernandez, 1997; Hernandez, 2007; Higinio Sánchez Gómez, 2006; Quintana, 1997; Romeo, 2009).

Packaging and pasteurization.

At the end of the production process, the olives are packaged and then pasteurized to prevent progressive deterioration. This operation involves a series of procedures. After the olives are rinsed with fresh water, they are lightly blanched using steam. Then, after sorting to remove any damaged or otherwise defective olives, the containers are filled. The concentration of the brine used for packaging can vary from 3% to 5%, and the pH value must be less than 4.6. After the containers are sealed, they are pasteurized at 90° C for about an hour. (Brighigna, 1998; Javier Casado, 2007; Javier Casado, 2010; Unal. & Nergiz, 2003).

2.1.2 The "Castelvetrano" method

The "Castelvetrano" method, using the olive-variety known as *"Nocellara del Belice"* (typically grown in the Castelvetrano area of Sicily, in Italy, after which the method is named), is used for preparing olives in soda. During this method of preparation, olives which have already been cleaned and size-graded are placed in a solution of water, soda (1.8 -2.5%) and salt (3-5 %). This procedure enables a rapid sweetening of the drupes, which retain a good consistency, a green colour and a distinctive taste of lye due to the NaOH solution (between 0.3% and 0.5%) which is left in the flesh. After 10 to 15 days of debittering treatment, the olives are subjected to a brief rinse using water or brine. The shelf-life of these olives is linked to their pH value, which after 2-3 months tends to go down and thus encourage the development of harmful microorganisms. After packaging, the product should be sterilized in an autoclave at temperatures of about 120° C, to prevent the formation of Clostridium Botulinum and the subsequent production of the Botulin toxin (Brighigna, 1998).

2.1.3 The method used for producing "Naturally-processed green olives"

"Naturally-processed green olives" can be prepared in various ways: whole, crushed, stoned, dressed or flavoured with spices. However, in all these cases the debittering process is carried out naturally, without any form of chemical intervention during the deamarization. In general, it takes at least 10-12 months of fermentation and storage in order to end up with a high-quality product, but there are some varieties of olive which are naturally sweet and require less time (Amelio & De Muro, 2000). The product is preserved in brine, the concentration of which is kept stable between 8% and 10%. The length of the sweetening process depends on several factors such as olive-variety, the amount of oleuropein (a bitter glycoside) in the fruits, the ripeness of the olives and the area where they were grown. The finished product is pleasantly bitter, with a slight winey taste due to the fermentation of the sugar components in the flesh of olives.

The only wastewaters produced during this "natural" fermentation are the rinsing water and the brine. In order to reduce pollution, the brine can be regenerated and re-utilized (Garrido-Fernandez, 1997; Quintana, 1997).

2.2 Black olives

Black olives, which are picked when they are almost fully ripe, may be processed by various methods. In general, the olive-varieties that are grown to be processed as black olives are those whose drupes have a thin peel, flesh with a good consistency and a very good colour, as well as a good flesh-to-pit ratio. When the olives are "naturally processed" in brine, the technology is not very different from that used for green olives (as described above), and the only real difference lies in the organoleptic characteristics of the finished product. However, while the method used for processing "Californian-style" black olives is similar to that used for "Spanish-style" green olives, the amount of alkali, the number of rinses and the colour-fixing stage are all different.

2.2.1 The "Californian-style" method (ripe table olives)

The product of this processing method, originally from California, is thus defined by the US Department of Agriculture: "Olives treated and oxidized during processing, in such a way that they assume a characteristic colour that ranges from dark brown to black, are called 'ripe table olives'". These olives, picked when partially or fully ripe, are first sorted and size-graded.

Storage in brine.

Before treatment with a NaOH solution, the olives are stored in brine at a concentration of between 8% and 10% for at least 30 days. The olives are preserved either in an acid solution (0.4% lactic acid) or by refrigeration (Gomez et al., 2006).

Lye treatment (Debittering)

The original processing method requires repeated debittering treatments (usually three) with a solution of 1%-2% sodium hydroxide, with each treatment lasting between 2 and 6 hours. During the rinsing phases, between one lye treatment and the next, insufflations of air into the water enable the olives to be thoroughly mixed. This helps to darken the surface of the fruit and encourages the enzymatic oxidation of the phenolic compounds present in it. These days the larger olive-processing firms, in order to simplify production, prefer a single treatment using a sodium solution at a concentration of between 1.2% and 1.5%, until the soda has completely penetrated the flesh of the drupes. Agitators or pumps are employed to mix the solution and prevent the soda collecting at the bottom of the container (Brenes, 2004; Higinio Sánchez Gómez, 2006).

The "Californian-style" method has a variant which uses only one debittering treatment with a lye solution at a concentration that ranges from 1.3% to 2.5%. The alkaline treatment is stopped when the lye has penetrated about 2/3 of the way into the flesh of the olives.

Rinsing between treatments

The rinsing, which has to be carried out between the various lye treatments, leads to a significant consumption of fresh water. As mentioned above, the rinses have the dual function of removing the sodium left in the olives and oxidizing them. Olives processed using the variant of this method (with its single debittering treatment) should undergo more rinses. It is also advisable to use lactic acid to sweeten the product and improve its chemical stability.

Immersion in a ferrous salt solution

After the lye treatment, the drupes are immersed for 12-24 hours either in a ferrous gluconate solution (1 - 2 g/L) or in a ferrous lactate solution (0.5-1 g/L). The aim of this phase is to give the olives a uniformly black colour which will be permanent (Brighigna, 1998; Garrido-Fernandez, 1997; Higinio Sánchez Gómez, 2006).

Final rinsing

After immersion in a ferrous salt solution, the olives are rinsed several times (from a minimum of 2 to a maximum of 8) until the rinsing water is pH neutral. Sometimes the water is heated to 80° C to prevent softening of the flesh and its consequent "fish eye" deterioration (caused by gas pockets). Once the rinsing water is pH neutral (after at least 2 rinses) there may be an optional phase of exposing the olives to air for 2-3 days, followed by a further size-grading.

Packaging and sterilization

These olives are usually packaged in brine with 2-5% NaCl and a pH of <4.6. The packaging procedures are basically the same as those for "Spanish-style green olives", described above. The only significant difference is the composition of the brine: in the "Californian-style" method this has a lower salt concentration and a higher pH value, which together create favourable conditions for the development of pathogenic germs and airborne or sporulate bacteria (such as Clostridium botulinum) - with serious consequences both for the consumer and for the product itself. It is therefore necessary to sterilize the containers by subjecting them to temperatures of 121° C for about an hour. After this heat treatment, samples from each batch should be subjected to microbiological controls in a specialized laboratory. It must be said, however, that sterilization affects the organoleptic properties of olives. Moreover, in some olives treated in this way, the flesh becomes less firm. (Kanavouras, 2005). The injection of calcium chloride (1 kg per 100 kg of olives) into the final wash may help to maintain the consistency of the drupes; any use of this additive, however, must be explicitly mentioned as 'E 509' on the label of the container.

3. Chemical characteristics of the wastewater from table olives processing

The production of table olives (green, naturally black etc.) involves various and consistent flows of wastewater (from min 0.5 liters/kg to 6 liters/kg). Every year, in the world from almost 1 million to almost 11.7 million tons of wastewater are generated from the processing of 1.9 million tons of table olives (assessment done by the author on the 2003-2009 average data coming from IOC, International Olive Council). Table 2 shows the production and assessment of the wastewater generated from the table olive processing, distinguishing for Country. The number of flows and their respective volumes are different and they depend of the kind of finished product. In order to have a picture more complete, the wastewater deriving from the washes of the container and those used for the packaging should be added to the amount indicate in the table 3. The processing of table olives dates back to a lot of years ago, so the industries never considered the availability of the water as resource and the environmental effects deriving from its use. The processing of table olives is an activity concentrated in a few months per year (in particular autumn-winter) and in restricted geographic areas (sometimes with little surface water resources). This determines strong pressure on the water resources and on the quality of the surface water.

Before that these problematic were pointed out with particular gravity, relevance wasn't been given to the surface sampling, or at the ground and at the draining modality of the wastewater. The growing attention towards the use of natural resources and the arising of the national laws and, in the case of Europe Union, of community laws ever more constricting for what concerning the draining of wastewater, involve the needing to assess with attention the modality of the water use into the table olives production processes.

	Countries										
	ES	EG	TR	SY	GR	MA	DZ	AR	USA	IT	Others
Production	503.3	299.6	230.8	143.3	108.2	98.3	81.9	80.8	79.7	65	249
Estimated wastewater (min)	252	150	115	72	54	49	41	40	40	32	125
Estimated wastewater (max)	3020	1798	1385	860	649	590	491	485	478	390	1494

Source: Author estimation based on data COI

Table 2. Table olives production and estimated waste water (average 2003-2009) (1,000 tonnes)

Table olive processing methods	**Lye**	**Fermentation brine**	**Washing**	**Preservation brine**	**Total**
Spanish style	0.5	0.5	0.5-2.0	0.5	2-3.5
Untreated green and turning colour olives		0.5		0.5	1
California green ripe olives	0.5	0.5	0.5-2.0	0.0-0.5	1.5-3.5
California black ripe olives	0.5-2.5	0.5	0.5-3.0	0.5	2-6.5
Naturally black olives		0.5		0.5	1

Source: Garrido-Fernandez et al, 1997

Table 3. Volume (L) of wastewater per kg of olives produced during the main phases of different types of table olives.

The problem can be cope with different and complementary ways:

- By trying to decrease the entire water requirement in the process (by reduction of the volume used in the single step of the process, exclusion of some operations from the process or reduction of its number, reuse of the same water on the same operation but on different batches of table olives;
- By trying to reduce the environmental impact of the wastewater (low percentage of NaOH in the lye or NaCl in the fermentation brine);
- By setting specific wastewater treatment methods.

The approaches listed above in point 1 and 2 are process improvements, while, the wastewater treatment is not affected by modifies applied to the processing method.

All these approaches were been studied in the last ten years. However, before to analyzing the most important results, it could be useful to watching the chemical characteristics of the wastewater generated by the various processes.

3.1 Wastewater from Spanish-style green olives processing

The approximate characteristics of the wastewaters from this treatment of the green olives are reported in the table 4.

General characteristics of wastewaters from processing Spanish-style pickled green olives in brine			
Characteristic	**Lye**	**Washing water**	**Fermentation brine**
pH	9.5 – 12.0	9.0 – 11.5	3.8 – 4.2
Free NaOH (g/L)	11.0	1.5	-
Free acidity (g lactic acid/L)	-	-	6 – 15
Polyphenols (g tannic acid/L)	2.5 – 4.0	2.5 – 4.0	4.0 – 6.0
Reducing sugars (g glucole /L)	6 -9	6 -9	-
Dissolved organic solids (g/L)	20 - 30	20 - 30	15 - 25
Dissolved inorganic solids (g/L)	20 - 35	7 - 25	90 - 110
COD (g O_2/L)	15 - 35	12 - 35	10 - 35
BOD_5 (g O_2/L)	9 - 20	9 - 20	8 - 20

Source: Garrido-Fernandez et al, 1997

Table 4. Characteristics of the wastewater from Spanish-style green olives processing

All these wastewaters are highly polluting and are not simply treated by conventional methods. As it can be seen, there are two different groups of wastewaters, alkaline (lye and washing waters) and acidic (fermentation brine). Their management and treatment need to be separate and different.

Lye and washing water noticeable differ in residual alkali concentration, and, in minor amount, in dissolved inorganic solids. The content of polyphenols is higher in the fermentation brine, while is null the one of reducing sugars.

Longer is the contact time between fruits and solution in each step greater are the dissolved substances. So, if it is good to dissolve the greatest amount of polyphenols, it is not good to extract reducing sugars during the lye treatment and washing. They are necessary during the successive fermentation step.

The brine′ main characteristic is the high concentration of inorganic components (mainly Na and K salts). They come from the added NaCl and from the high content of the olive flesh.

The contemporary presence of substances easily used by microbes as carbon sources (reducing sugars in lye and washing waters, lactic acid in brine where it accounts for about

80% of the BOD_5), and substances that interfere with them (polyphenols), seriously interfere with the direct application of any biological purification procedure.

In conclusion, these wastewaters are heavily polluted, perhaps difficult to treat and dispose of. Many strategies have been studied to reduce the environmental impact of these wastewaters: internal control measures, such as lye and washing waters re-use, reduction or elimination of washing waters, debittering with low-concentration lyes, regeneration and re-use of fermentation brine. Any of these approaches has completely resulted in meeting the needs (Garrido-Fernandez et al., 1997).

Table 5 shows the value, expressed in grams per litre of oxygen, biological oxygen demand (BOD_5) and chemical oxygen demand (COD) of wastewater processing from Spanish-style.

Wastewater	pH	BOD_5 (g O_2/L)	COD (g O_2/L)	NH_3 (mg/L)	B (mg/L)	P (mg/L)	Fe (mg/L)	Cu (mg/L)	Zn (mg/L)	Ni (mg/L)	Hg (mg/L)	Na (g/L)	K (g/L)	Cl- (mg/L)
Lye	12.7	4.8	24.0	1.3	0.14	18.3	5.75	0.23	0.48	0.12	0.08	96.5	18.5	2.9
1st Washing	9.1	3.1	10.0	16.8	0.63	28.4	3.62	0.16	0.36	0.09	0.05	15.7	4.2	1.9
2nd Washing	7.6	1.6	7.6	1.3	0.40	16.3	1.54	0.10	1.64	0.03	0.05	10.5	2.6	1.1
3rd Washing	6.2	0.9	3.0	2.0	0.34	11.4	0.84	0.06	0.08	0.02	0.09	5.0	1.7	0.7
4th Washing	6.5	0.6	2.0	2.3	0.28	10.6	0.82	0.04	0.07	0.01	0.06	3.7	1.1	0.5
5th Washing	7.0	<0.1	0.2	3.5	0.16	4.8	0.40	<0.01	0.12	0.01	0.05	1.1	0.3	0.2

Source: Garrido-Fernandez et al, 1997

Table 5. Loads polluting wastewater processing of green olives by the "Spanish-style"

3.2 Wastewater from turning colour olives processing

Processes not based on an alkaline treatment, comprising only fermentation or storage brine and, sometimes, water from washing before packing, produce a less volume of wastewaters that are less contaminated.

Main wastewaters from a typical darkening stage								
Characteristic	**Holding brine**	**1st treatment**		**2nd treatment**		**3rd treatment**		**Gluconate solution**
		Lye	**Wash**	**Lye**	**Wash**	**Lye**	**Wash**	
pH	-	12.11	6.90	11.85	8.00	11.70	8.55	3.70
Free NaOH (g/L)	-	8.90	-	4.60	-	4.30	-	-
Dissolved inorganic solids (g/L)	-	2.38	3.18	4.34	7.21	6.88	8.14	7.33
Dissolved organic solids (g/L)	-	19.27	3.15	12.25	32.21	12.08	36.64	43.38
Polyphenols (g/L)	-	0.18	0.22	0.59	0.31	0.48	0.64	0.80
COD (g O_2/L)	35.0	2.0	3.5	2.5	3.8	2.6	3.7	1.5

Table 6. Characteristics of the wastewater from turning color olives processing

Process that includes alkali treatment and the darkening stage (by the use of ferrous gluconate) (California-style method), needs a greater volume of water, due mainly to the numerous lye treatments and washes (Table 6).

It can be seen that the highest pollutant charge comes from the holding brine, while lyes and washing waters are relatively moderate. Fermentation-storage brine has characteristics very similar to those from Spanish-style green olives (Garrido-Fernandez et al, 1997).

3.3 Wastewater from naturally black olives processing

Fermentation brines are the only waste liquids produced during this type of processing that pose some problem. They are liquids with a high level of organic matter (Table 7).

Characteristics	Brines from different origins
pH	3.6 - 4.4
Salts (g NaCl/L)	56.0 - 77.0
Dissolved organic solids (g/L)	95.3 - 118.8
BOD_5 (g O_2/L)	34.8 - 38.3
Polyphenols (g/L)	3.2 - 5.1

Table 7. Characteristics of the wastewater from naturally black olives processing

It is the highest between all types of table olives processing, very close to those reached in olive oil mill wastewaters. The molecules contained in the organic matter of these brines, however, are only heavy potential contaminants, as shown by their high BOD_5 .

The salt content is higher than in any other process. Such concentrations can cause serious problems for conventional municipal wastewater treatment facilities.

3.4 Wastewater from black olives by Californian - style

Tables 8 and 9 show the values of BOD_5 and COD of wastewater processing of black olives by Californian-style and its variant, table 10 the values of TOC of packing brines of Spanish-style and Californian-style, and Table 11 the characteristic of Californian style wastewater.

Wastewaters	BOD_5 (mgO_2/L)	COD (mgO_2/L)
Preservation	10,620	35,000
1st Lye treatment	607	2,000
1 st Washing	1,062	3,500
2nd Lye treatment	759	2,500
2nd Washing	1,153	3,800
3rd Lye treatment	789	2,600
3rd Washing	1,123	3,700
Immersion in ferrous salt solution	455	1,500
4 th Washing	394	1,995
5th Washing	394	1,995

Source: Garrido-Fernandez et al, 1997

Table 8. Pollution loads of wastewater from processing of black olives processed by the Californian style

Wastewaters	**BOD$_5$ (mgO$_2$/L)**	**COD (mgO$_2$/L)**
1 st Lye treatment	150	4,260
1 st Washing	230	6,210
2 nd Washing	200	3,880
3 rd Washing	190	1,480
4 th Washing	190	1,680
5 th Washing	294	2,950
6 th Washing + lactic acid	336	5,550
7 th Washing + lactic acid	200	5,840
8 th Washing + lactic acid	434	5,580
Immersion in ferrous salt solution	313	6,650
9 th Washing	430	2,320
10 th Washing	357	1,670

Table 9. Pollution loads of wastewater from black olive processing by variant of the Californian style

Preservation brine	**Green olives**	**Black olives**
TOC (mg/L)	3,089	3,368

Table 10. Loads of pollutants brines packaging, expressed as total organic carbon (TOC)

"Californian Style" wastewater	
Method A	**COD**
1 st Lye treatment	2,000
1 st Washing	3,500
2 nd Lye treatment	2,500
2 nd Washing	3,800
3 rd Lye treatment	2,600
3 rd Washing	3,700
Immersion in ferrous salt solution	1,500
4 th Washing	1,995
5 th Washing	1,995

Source: Garrido-Fernandez et al, 1997

Table 11. Characteristic of Californian style wastewater

4. Pollution prevention methods in the table olive industries

Prevention is better than curing. So internal control measures of potential pollutants or water use must be preferred as the first choice to tackle the environmental impacts of these industries.

Internal control measures include:

- Re-use of lye, washing waters, fermentation/storage brine;
- Reduction or elimination of washing waters;
- Debittering with low-concentration lyes;
- Salt-free storage solution.

Notwithstanding many experimental studies fulfilled during the latter decades, no one has produced results totally sharable, so, not even one was introduced as process improvement. This because, each modify, introduced respect to the traditional process, ever involves variations in the quality of the product which aren't accepted. As was noted, the chemical composition of the table olive wastewaters limits the effectiveness of classical purification systems. Polyphenols (oleuropein and derivates, anthocyanins) and sodium content (from the NaOH in the lye and from NaCl in the brines) are the most important pollutants contained.

Accordingly, in the latter years a number of studies were carried out in order to adapt these wastewaters to the limits accepted by the national environmental laws (Deligiorgis et al., 2008).

Purification (partial) of lyes and washing waters from green table olives processing by lowering the pH under the neutrality and by leading the temperature of the wastewater around 0° C (De Castro et al., 1983), allow to bring COD down over 35%. The wet oxidation carried out in alkaline environment into a pressurized reactor, at the temperature of 200° C, is able to eliminate almost all over the polyphenols, and if the pure oxygen is used, also the 90% of the organic carbon present at beginning.

The efficiency is lower when the same methods are applied to the washing waters from the darkening process of black-ripe olives. Fermentation brine represents more than the 22% of the total final wastewaters from green table olives processing, but account for approximately 70% of the organic pollution charge. Thus, if these brines are correctly purified much of the pollution potential is eliminated. Physicochemical treatment reduce the pollutant charge (COD) of the fermentation brines only about 20÷25%.

There is not much experience of biological treatment to remove the excess organic charge up to now (Beltran et al., 2008). The main problem is related to polyphenols degradation and the salt level; appropriate dilution and/or adapted microorganisms are needed. *Brenes et al.* showed that an activated sludge process can be used successfully, yealding a 80% COD reduction, but only a small proportion of polyphenols was consumed. A manner to reduce the polyphenols content could be the wet air oxidation (Brenes et al., 2000; Beltràn et al., 2000a; Katsoni et al., 2008).

The best results were obtained at acidic pH, catalized by means of Cu_2+. The addition of H_2O_2 in the absence of copper resulted in a lower COD conversion, while an increase in the biodegradability of the final mixture was found after having added the radical promoter.

Kotsou et al. studied an aerobic biological treatment using an Aspergillus niger strain, in combination with chemical oxidation (H_2O_2), followed by coagulation with CaO of the resulting treated waters. The results of the experiments are shown in the Table 12 (Kotsou et al., 2004).

The main effect of the chemical oxidation step was the elimination of persistent phenolic compounds during the biological treatment of total phenolic compounds.

Influent		After the biological step HT 2 days		After chemical Oxidation [H_2O_2] 4g/L [Fe^{2+}] 0.5 g/L		Affluent after liming CaO 5 g/L Sed. Time 2h	
COD mg/L	Phenols mg/L	COD mg/L	Phenols mg/L	COD mg/L	Phenols mg/L	COD mg/L	Phenols mg/L
11000	190.7	3950	62.4	1537	32.4	835	5.4
11130	185.5	4043	64.7	1625	29.5	825	5.1
9300	150.8	3575	38.1	1327	25.7	790	4.8
8500	147.9	3616	39.6	1452	17.4	890	4.3
20920	319.5	5185	187.5	2225	63.2	1675	21.2
9750	157.8	3275	30.7	1297	27.3	675	4.3
9080	152.2	2135	33.2	1050	16.9	780	4.6
8190	137.4	2700	32.3	1027	11.8	795	3.4
9960	178.3	2160	41.6	1097	20.1	835	3.8
9850	185.3	2227	37.4	1105	22.7	895	3.5

Source: Kotsou et al., 2004

Table 12. Characteristics of the wastewater after aerobic biological treatment

Beltran et al. studied the purification efficacy of the aerobic and anaerobic biodegradation of the wastewater from green table olive processing using acclimatized bacterial flora taken from, respectively, an activated sludge from a municipal wastewater treatment plant and a biomass from an anaerobic digester of a municipal wastewater treatment plant.

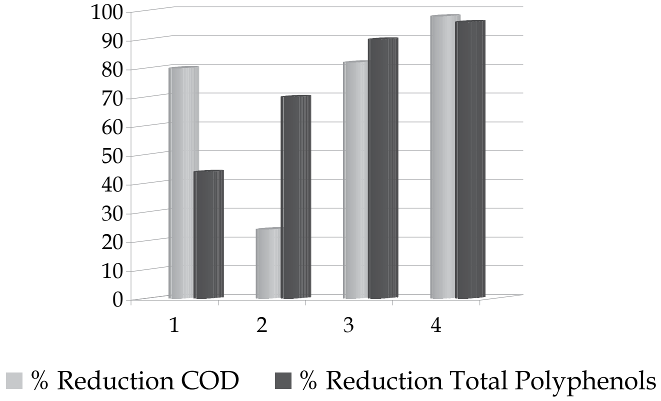
The aerobic biodegradation a significative reduction of the COD between 50% and 70%, and an important removal of the total polyphenolic compounds around 97%; the anaerobic digestion achieves an important removal of COD between 81 and 94%, indicating that most of the substrate fed to the digester is biodegraded anaerobically (Beltran et al., 2008).

Different chemical oxidants, alone and combined, were applied to the purification of the wastewaters from the storage in brine of black table olives. The most effective purification process was the overall combination O_3/UV/ H_2O_2. Aerobic treatment of the effluents gave a major substrate removal that was independent of the initial biomass concentration. The chemical treatments in general, and ozonation processes in particular, are useful for the degradation of organic matter, especially aromatic compounds.

These processes can be used as pre-treatment steps for subsequent aerobic degradation in order to meet discharge norms and reach purification efficiencies required by national regulations (Beltran-Heredia et al., 2000a; Beltran-Heredia et al., 2000b; Beltran-Heredia et al., 2000c; Beltrán de Heredia et al., 2001d; Beltrán de Heredia et al., 2001a; Beltrán de Heredia et al., 2001b; Benitez et al., 2003).

The figure 3 shows the results obtained in experiments carried out on the wastewater of black ripe table olives which foresaw only the aerobic biologic treatment (1), the only treatment by ozone (2), the treatment by ozone followed by a aerobic biologic treatment (3), the aerobic biologic treatment followed by the ozone treatment (4).

Figure 3 shows that the biologic treatment is more efficient in order to reduce the COD, while the ozone is more efficient in the reduction of the polyphenols. The two treatments employed one after the other allow to bring down almost 90% of COD and polyphenols. The wastewater treated are odorless, uncolored and disinfected. They could be reintroduced into another productive loop.



Source: Beltran-Heredia, 2001

Fig. 3. Results obtained by ozone treatment on the wastewater of black ripe table olives

Instead of to eliminate the polyphenols from the washing water, an alternative washing process could be that which foresee the recovery of the substances useful from an economic point of view (e. g. hydroxytyrosol) (De Castro & Brenes, 2001; Bouaziz et al., 2008).

A similar proposal, but referred to the exhausted lyes deriving from the deamarization phase of the various table olive processing methods, is in the research project of D'Annibale et al. "New technology in virgin olive oil mechanical extraction process in relationship with the possible traceability and nutritional and sensory quality of oil and exploitation of vegetation waters and pomaces by recovery the bioactive products" (D'Annibale et al., 2005). This proposal is enriched from the proposition to produce enzymes from brines in order to create an economic interest. When, for various reasons, it is impossible to apply some wastewater depuration treatment able to bring down the pollution in order to dispose of it in agreement with laws, in the warm regions in which the weather is windy and there is drought, an extreme remedy could be the lagoon. In this case great attention should be given to the bad smells and leaching risk. The evaporation can be increased, favouring the absorption of the sun radiation into the lagoon basin (Chatzisymeon et al., 2008), by using surfactant agents, re-circle and spraying.

5. Life Cycle Assessment (LCA) methodology to assess the impact of wastewater of table olives industries

5.1 About Life Cycle Assessment

The LCA (Life Cycle Assessment), is a valuable analysis tool, potentially applicable to any product, process or service, and it is subject to specific standards by the International Standardization Organization (ISO). The rules of the ISO 14040 series of the Environmental Management Life Cycle Assessment (four standards dedicated each one to a specific part of the methodology), it is the point of reference for the application, in business decisions, of this environmental management methodology (ISO 14040, 2006; ISO 14044; ISO /TR 14048).

The LCA can be used as a technical tool to identify and assess opportunities to reduce the environmental effects associated with specific products, production processes, packaging, materials or activities or to identify various scenarios on which to make strategic choices. This tool can also be used to achieve a sustainable management of the natural resources. This methodology, adopting a systemic approach, can be used as tool of private or public decisions in the various productive choices during a definition of environmental standards, to establish the basis of information from a single stage of the production process up to chain, to identify "process hot spots", as a product certification tool (for example Eco-label).

The LCA, considers the product as a system, that is analyzing the changes and flows of matter and energy, since their withdrawal from national system, that of production of the product, until the final disposal.

Numerous LCA studies were focused on the olive oil chain (Notarnicola et al., 2004; Olivieri et al., 2007; Raggi et al., 2000; Olivieri et al., 2005; Russo et al., 2008), but not many on the table olive chain (Cappelletti et al., 2010), however each business reality presents different characteristics (Salomone et al., 2010).

5.2 The goal and scope definition

The Life Cycle Assessment methodology was applied to the various methods used to process green and black table olives.

As for the green table olives the processes analysed were: Spanish- style, Castelvetrano-style and Green natural-style, while, as far as the black table olives, two methods of the California black ripe olives were studied (Russo et al., 2010). This analysis aims to highlight the contribution, of the wastewater produced from each processing method, to the totally environmental burden referred to the table olives industries. For this reason the system boundaries were simplified including only the sub-phases referred to the processing methods. In order to better focusing the study on the industrial phase, the agricultural phase and packaging were excluded from the analysis. The Functional Unit (FU) chosen was 100 kg of fresh table olives.

5.3 The Life Cycle Inventory (LCI)

As for the input and output concerning the processing methods, all the relevant data were considered. Although in different quantity, Spanish-style (figure 4) and Castelvetrano-style (figure 5) have the same input, while the input of green natural-style (figure 8) are quite different for quantity and type respect to these of the other methods used to processing green table olives. As for the methods used to processing black olives, the two methods of California black ripe olives present same input (figure 6 and 7), but also in this case the amount is not the same. As for the output, the amount of waste are the same for the green olives processes and quite high for the two methods of California black ripe olives. As far as the wastewater, figure 9 shows the quantity produced by each processing methods; among the various processes, the method B of the California black ripe olives produces the greater amount of wastewater, almost 9 kg per kg of fresh raw material. This is due to the lower quantity of caustic soda used respect the method A and the higher number of washes needed to oxidizing the olives and fixing their black colour.

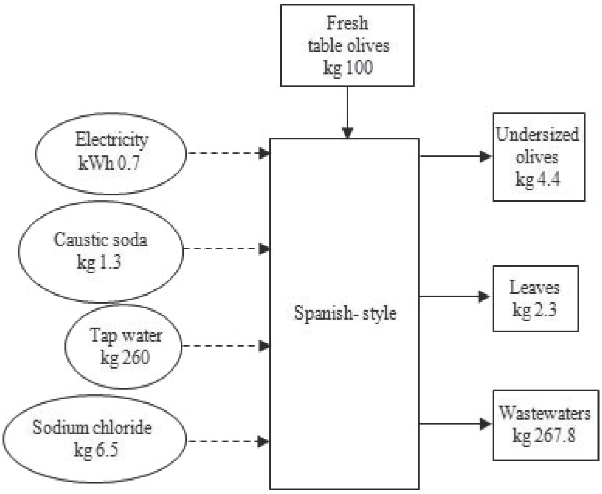


Fig. 4. Layout of Spanish-style

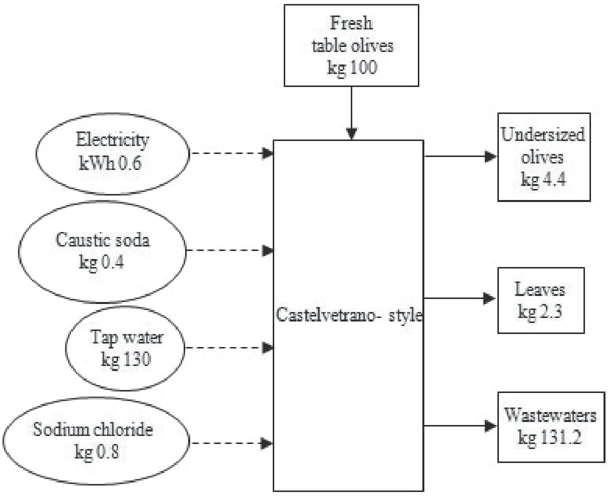


Fig. 5. Layout of Castelvetrano-style

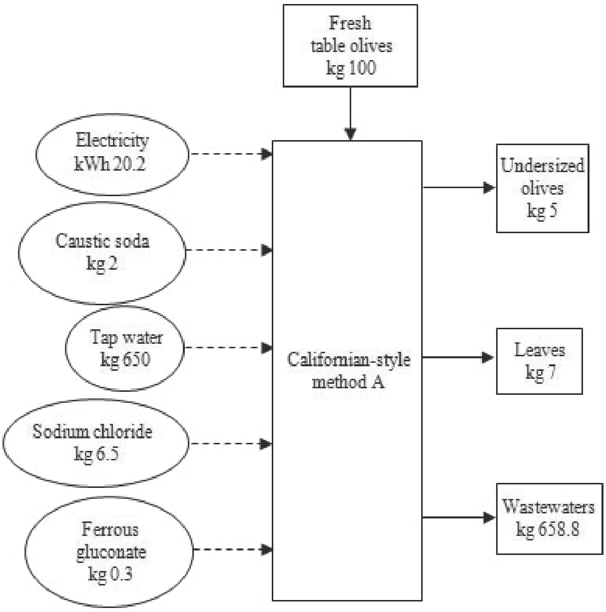


Fig. 6. Layout of California black ripe olives method A

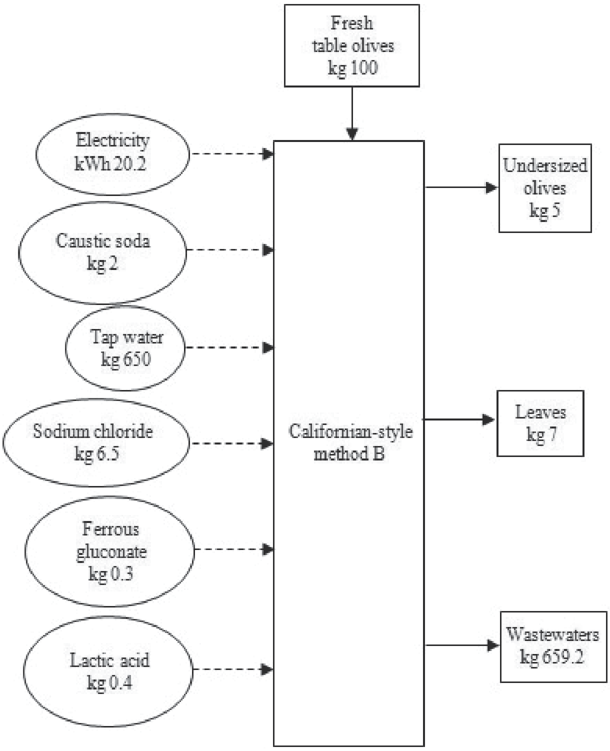


Fig. 7. Layout of California black ripe olives method B

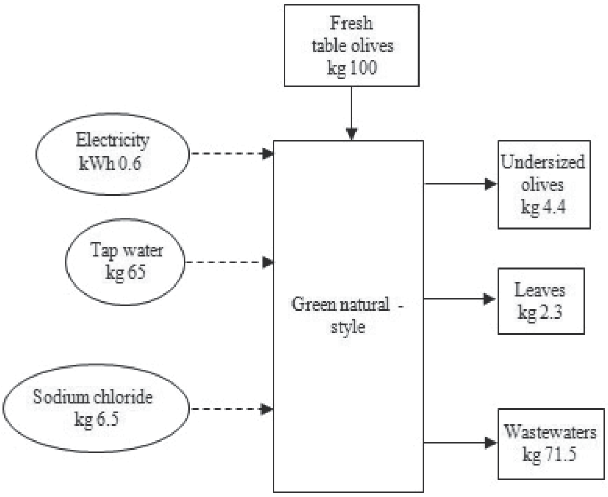


Fig. 8. Layout of the green natural-style

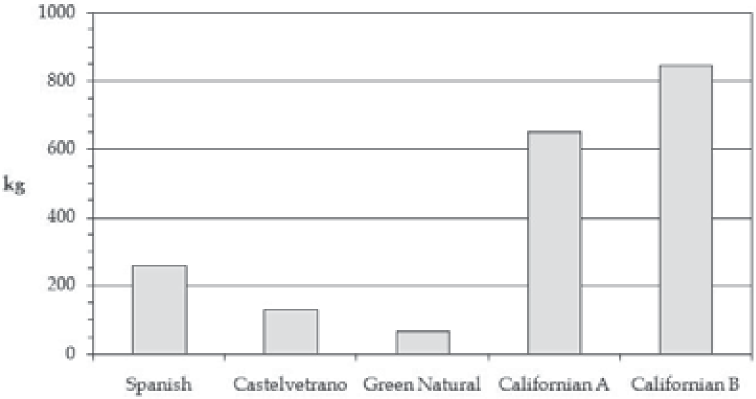


Fig. 9. Wastewater referred to 100 kg of processed table olives, distinguishing among the various manufacturing processes

In order to better compare the amount of materials and energy and the waste produced by the processing methods considered, table 13 shows, for each one, the amount of all the input and output. In particular, as for the wastewater, these are distinguished for each sub-phases. Among the methods used to processing green olives, the Spanish-style is the one that needs the higher amount of water, electricity and caustic soda, while comparing all the processes, the method B of the California black ripe olives needs more quantity for almost all the input (exception for caustic soda, employed in higher quantity in the California black ripe olives method A). Focusing the attention on the production of wastewater, table 1 shows that, exception for the green natural style, the sub-phase in which wastewater are produced in greater quantity is the washing phase. It is due to the need to eliminate the residues of caustic soda after the deamarization phase. The green natural-style don't employ caustic soda because of the deamarization take place in a solution of water and salt and after the olives don’t need to be washed, but this method take a long time and not for all the variety of table olives is convenient from an economic point of view.

		Green table olives			**Black table olives**	
Input		**Spanish style**	**Castelvetrano style**	**Green Natural -style**	**California black ripe olives method A**	**California black ripe olives method B**
Water	kg	260.0	130.0	65.0	650.0	845.0
Electricity	kWh	0.7	0.6	0.6	20.2	27.3
Sodium chloride NaCl	kg	6.5	0.8	6.5	6.5	6.5
Lactic Acid	kg					0.4
Caustic Soda NaOH	kg	1.3	0.4		2.0	1.3
Ferrous Gluconate	kg				0.3	0.3
Output						
Undersized Olives and Leaves	kg	6.7	6.7	6.7	12.0	12.0
WW Preservation in Brine	kg				71.5	71.5
WW Deamarization	kg	66.3	66.2	71.5	197.0	66.3
WW Washes	kg	130.0	65.0		325.0	650.0
WW Fermentation	kg	71.5				
WW Ferrous gluconate attack	kg				65.3	65.3

Table 13. Input and output referred to the various table olives manufacturing processes

5.4 Data quality

The inventory data was collected from various sources. The data concerning the input of technological processing was collected directly from processing companies which produce green table olives, by using Spanish-style, and black-ripe table olives, by using California black ripe olives method A and B. Electricity consumption was measured, as well as the amount of input resources and wastewater. For what concerning the green natural-style and Castelvetrano-style, also some data coming from literature was considered (Garrido-Fernandez et al., 1997). As far as electricity production, the Italian mix was the type considered (AEEG, 2010). As far as the issues of wastewater; by means of laboratory analyses we assessed the pollution caused by the exhausted brines, lyes and washing waters referred to the Spanish-style and California black ripe olives, while for green natural-style and Castelvetrano-style we considered literature data. All collected data was processed by using GaBi4 software and its databases (IKP and PE, 2002), also Ecoinvent database was used (Frischknecht et al., 2004).

5.5 LCIA (Life Cycle Impact Assessment)

The CML 2001 impact assessment method was used to analyse the environmental impact of the input and output measured during the inventory phase. The categories of pollution considered by this method were: abiotic depletion potential (ADP), acidification potential

(AP), eutrophication potential (EP), freshwater aquatic ecotoxicity potential (FAETP inf.), global warming potential (GWP 100 years), human toxicity potential (HTP), marine aquatic ecotoxicity potential (MAETP), ozone layer depletion potential (ODP, steady state), photochemical ozone creation potential (POCP), radioactive, radiation (RAD) and terrestric ecotoxicity potential (TETP). All these impact categories were opportunely weighted and normalized in order to obtain an eco-indicator.

Figure 10 shows, for each processing method, the value of the CML 2001 eco-indicator deriving from the sum of the normalized value of the impact categories. As for the methods used to processing green olives, the category EP is the most important, while as far as the California black ripe olives the category that mainly contribute to the total eco-indicator is MAETP, but also EP and GWP increase the value. This is due to the impact deriving from the greater use of the electricity required to oxidize the olives and fixing their black colour.

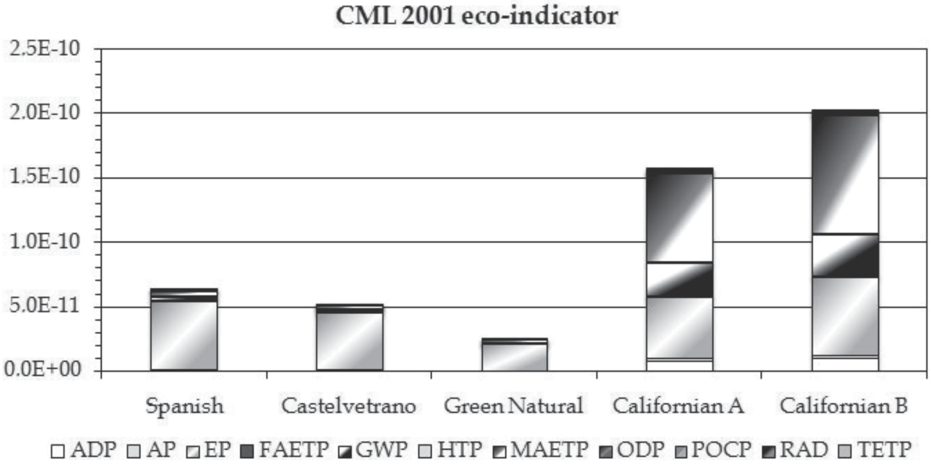


Fig. 10. Eco-indicator referred to the various table olives manufacturing processes

The LCA results underlined that the eutrophication is a very important impact for the table olives processing industries, and it derives from the pollution of the wastewater. In order to better understand the pollution of the wastewater of table olives industries, figure 11, shows, for each processing method the contribution to the eutrophication of the wastewater produced in the different sub-phases of the process.

By analysing the Spanish-style, the exhausted brine is more pollutant than lye, while, even though the washing waters are greater in volume, they are the less pollutant, the deamarization phase of the Castelvetrano-style is quite different from that of Spanish-style because sodium chloride is added to the lye, in order to accelerate the deamarization process, so, as shown in figure 11, the wastewater from this phase have higher environmental burdens, the washing waters further affect the eutrophication, but the total value is lower than the one referred to the Spanish-style.

As for green natural style, the process don’t foresee washes but only deamarization in brine, so the wastewater produced in this phase are the only contributor to the EP and their pollution, among all the processes analysed is the lowest.

By analysing the California black ripe olives, figure 11 shows that as for the EP an important contribution is due to the wastewater deriving from the phase of preservation in brine, also the washing waters heavily contribute to the total EP, especially in the method B, while the

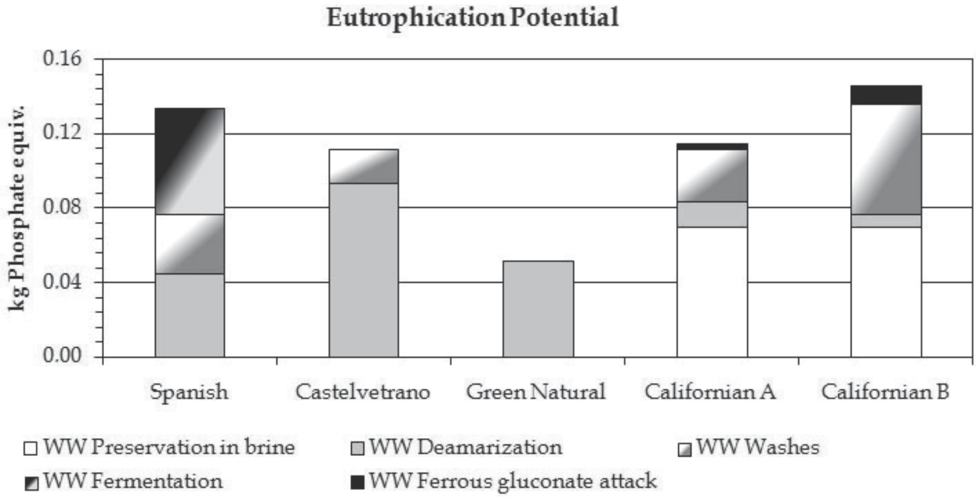


Fig. 11. Eutrophication Potential, distinguishing among the various table olives manufacturing processes

exhausted lyes and the wastewater deriving from the phase of ferrous gluconate attack present a low environmental impact.

Finally, even though the processes compared make use of different technologies to obtain end products each with distinct organoleptic characteristics, we can state that "Green natural style" processing gives a better environmental performance in terms of eutrophication potential and the California black ripe olives method B appears to be the most polluting overall, but it is relevant to point out that method A of California black ripe olives has a lower EP value than Spanish-style.

5.6 Improvement hypothesis

The Life Cycle Assessment has highlighted that the wastewater is an important issues deriving from table olives industries, and the impact category mainly affected by the pollution of wastewater is the eutrophication. Among the various processing methods used to process green and black table olives, wastewater are produced in different amount and affect in different way the impact category EU.

In order to improve the environmental performance of these processing–system, however, technological solutions could be adopted with the aim of reducing the amount of material used (caustic soda, sodium chloride, water), and consequently limit the negative effects connected to its production and use (Marsilio et al., 2008). It is also worth considering reusing the brine and the rinsing-water, in order to reduce the amount of wastewater produced and the emissions (Segovia-Bravo et al., 2008). An important improving solution could be also represented from the extraction of the useful substances, such as phenols, responsible to the high pollution of the wastewater (De Castro et al., 1983; Garrido-Fernandez, 1983; Garrido Fernandez et al., 1997; Bouaziz et al., 2008). Finally, especially for the Californian methods that use greater amount of electricity, environmental improvement should concern also the minimizing of the impacts deriving from energy production. This objective could be achieved by, e.g., installing renewable energy sources at the factory in order to use the electricity directly for these uses. It is important to consider that the adoption of these solution made with regard to achieving the objectives of eco-compatibility, leads to advantages, which are not only environmental (a lower consumption of resources, a reduction in pollutants etc.) but also economic (i.e. lower costs).

Contribution of authors

This paper has been thought, discussed and written by the three authors and it is the result of their common commitment, in particular Giulio Mario Cappelletti has contributed to paragraphs 1 and 2; Giuseppe Martino Nicoletti has contributed to paragraphs 3 and 4; Carlo Russo has contributed to paragraph 5.

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Part 3

Waste Water Management and Environmental Policies

20

Wastewater Management

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1. Introduction

Wastewater is water whose physical, chemical or biological properties have been changed as a result of the introduction of certain substances which render it unsafe for some purposes such as drinking. The day to day activities of man is mainly water dependent and therefore discharge 'waste' into water. Some of the substances include body wastes (faeces and urine), hair shampoo, hair, food scraps, fat, laundry powder, fabric conditioners, toilet paper, chemicals, detergent, household cleaners, dirt, micro-organisms (germs) which can make people ill and damage the environment. It is known that much of water supplied ends up as wastewater which makes its treatment very important. Wastewater treatment is the process and technology that is used to remove most of the contaminants that are found in wastewater to ensure a sound environment and good public health. Wastewater Management therefore means handling wastewater to protect the environment to ensure public health, economic, social and political soundness (Metcalf and Eddy, 1991).

1.1 History of wastewater treatment

Wastewater treatment is a fairly new practice although drainage systems were built long before the nineteenth century. Before this time, "night soil" was placed in buckets along streets and workers emptied them into "honeywagon" tanks. This was sent to rural areas and disposed off over agricultural lands. In the nineteenth century, flush toilets led to an increase in the volume of waste for these agricultural lands. Due to this transporting challenge, cities began to use drainage and storm sewers to convey wastewater into waterbodies against the recommendation of Edwin Chadwick in 1842 that "rain to the river and sewage to the soil". The discharge of waste into water courses led to gross pollution and health problems for downstream users.

In 1842, an English engineer named Lindley built the first "modern" sewerage system for wastewater carriage in Hamburg, Germany. The improvement of the Lindley system is basically in improved materials and the inclusion of manholes and sewer appurtenances—the Lindley principles are still upheld today. Treatment of wastewater became apparent only after the assimilative capacity of the waterbodies was exceeded and health problems became intolerable. Between the late 1800s and early 1900s, various options were tried until in 1920, the processes we have today were tried. Its design was however empirical until midcentury. Centralized wastewater systems were designed and encouraged. The cost of wastewater treatment is borne by communities discharging into the plant.

Today there have been great advances to make portable water from wastewater. In recent times, regardless of the capacity of the receiving stream, a minimum treatment level is required before discharge permits are granted (Peavy, Rowe and Tchobanoglous, 1985). Also presently, the focus is shifting from centralized systems to more sustainable decentralized wastewater treatment (DEWATS) especially for developing countries like Ghana where wastewater infrastructure is poor and conventional methods are difficult to manage (Adu-Ahyia and Anku, 2010).

1.2 Objectives of wastewater treatment

Wastewater treatment is very necessary for the above-mentioned reasons. It is more vital for the:

Reduction of biodegradable organic substances in the environment: organic substances such as carbon, nitrogen, phosphorus, sulphur in organic matter needs to be broken down by oxidation into gases which is either released or remains in solution.

Reduction of nutrient concentration in the environment: nutrients such as nitrogen and phosphorous from wastewater in the environment enrich water bodies or render it eutrophic leading to the growth of algae and other aquatic plants. These plants deplete oxygen in water bodies and this hampers aquatic life.

Elimination of pathogens: organisms that cause disease in plants, animals and humans are called pathogens. They are also known as micro-organisms because they are very small to be seen with the naked eye. Examples of micro-organisms include bacteria (e.g. *vibro cholerae*), viruses (e.g. enterovirus, hepatits A & E virus), fungi (e.g. *candida albicans*), protozoa (e.g *entamoeba hystolitica, giardia lamblia*) and helminthes (e.g. *schistosoma mansoni, asaris lumbricoides*). These micro-organisms are excreted in large quantities in faeces of infected animals and humans (Awuah and Amankwaa-Kuffuor, 2002).

Recycling and Reuse of water: Water is a scarce and finite resource which is often taken for granted. In the last half of the 20th century, population has increased resulting in pressure on the already scarce water resources. Urbanization has also changed the agrarian nature of many areas. Population increase means more food has to be cultivated for the growing population and agriculture as we know is by far the largest user of available water which means that economic growth is placing new demands on available water supplies. The temporal and spatial distribution of water is also a major challenge with groundwater resources being overdrawn (National Academy, 2005). It is for these reasons that recycling and reuse is crucial for sustainability.

1.3 Types of wastewater

Wastewater can be described as in the figure below.

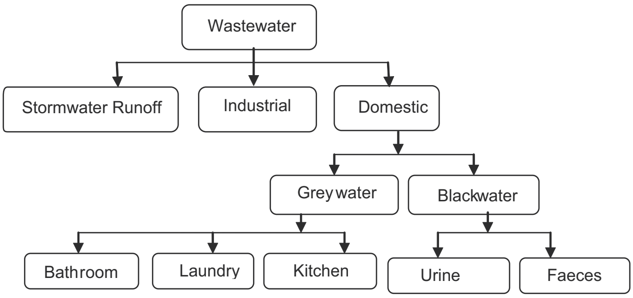


Fig. 1. Types of Wastewater

2. Definition of concepts and terminology

Stormwater Runoff is water from streets, open yard etc after a rainfall event which run through drains or sewers.

Industrial wastewater is liquid waste from industrial establishments such as factories, production units etc.

Domestic wastewater also known as municipal wastewater is basically wastewater from residences (homes), business buildings (e.g. hotels) and institutions (e.g. university). It can be categorized into greywater and blackwater.

Greywater also known as sullage is liquid waste from washrooms, laundries, kitchens which does not contain human or animal excreta.

Blackwater is wastewater generated in toilets. Blackwater may also contain some flush water besides urine and faeces (excreta). Urine and faeces together is sometimes referred to as night soil.

Sewage is the term used for blackwater if it ends up in a sewerage system.

Septage is the term used for blackwater if it ends up in a septic tank.

Sewerage system is the arrangement of pipes laid for conveying sewage.

Influent is wastewater which is yet to enter in a wastewater treatment plant or liquid waste that is yet to undergo a unit process or operation.

Effluent is the liquid stream which is discharged from a wastewater treatment plant or discharge from a unit process or operation.

Sludge is the semi-solid slurry from a wastewater treatment plant.

On-Site System: this is wastewater disposal method which takes place at the point of waste production like within individual houses without transportation. On- site methods include dry methods (pit latrines, composting toilets), water saving methods (pour-flush latrine and aqua privy with soakage pits and methods with high water rise (flush toilet with septic tanks and soakage pit, which are not emptied).

Off-Site System: in this system, wastewater is transported to a place either than the point of production. Off- site methods are bucket latrines, pour-flush toilets with vault and tanker removal and conventional sewerage system.

Conventional sewerage systems can be combined sewers (where wastewater is carried with storm water) or separated sewers.

Septic Tank is an on-site system designed to hold blackwater for sufficiently long period to allow sedimentation. It is usually a water tight single storey tank.

Faecal sludge refers to all sludge collected and transported from on-site sanitation systems by vacuum trucks for disposal or treatment.

Unit Operation: this involves removal of contaminants by physical forces.

Unit Process: this involves biological and/or chemical removal of contaminants.

Wastewater Treatment Plant is a plant with a series of designed unit operations and processes that aims at reducing certain constituents of wastewater to acceptable levels.

3. Characteristics of wastewater

Depending on its source, wastewater has peculiar characteristics. Industrial wastewater with characteristics of municipal or domestic wastewater can be discharged together. Industrial wastewater may require some pretreatment if it has to be discharged with domestic wastewater. The characteristics of wastewater vary from industry to industry and therefore would have different treatment processes—for example a cocoa processing company may have a skimming tank in its preliminary treatment stage to handle for instance spilt cocoa butter while a beverage plant may skip this in the design. In general, the contaminants in wastewater are categorized into physical, chemical and biological. Some indicator measured to ascertain these contaminants include (Peavy, Rowe and Tchobanoglous, 1985 & Obuobie et al., 2006):

Physical

- **Electrical Conductivity** (EC) indicates the salt content
- **Total Dissolved Solids** (TDS) comprise inorganic salts and small amounts of organic matter dissolved in water
- **Suspended solids** (SS) comprises solid particles suspended (but not dissolved)in water

Chemical

- **Dissolved Oxygen** (DO) indicates the amount of oxygen in water
- **Biochemical oxygen demand** (BOD) indicates the amount of oxygen required by aerobic microorganisms to decompose the organic matter in a sample of water in a defined time period.
- **Chemical oxygen demand** (COD) indicates the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant
- **Total Organic Compound** (TOC)
- **NH4-N** and **NO3-N** show dissolved nitrogen (Ammonium and Nitrate, respectively).
- **Total Kjeldhal Nitrogen** is a measurement of organically-bound ammonia nitrogen.
- **Total-P** reflects the amount of all forms of phosphorous in a sample.

Biological

- **Total coliforms** (TC) is encompassing faecal coliforms as well as common soil microorganisms, and is a broad indicator of possible water contamination.
- **Faecal coliforms** (FC) is an indicator of water contamination with faecal matter. The common lead indicator is the bacteria *Escherichia coli* or *E. coli.*
- **Helminth** analysis looks for worm eggs in the water

3.1 Process of wastewater treatment

Due to the nature of contaminants in wastewater—physical, chemical and biological, the unit operations and processes in wastewater treatment can also be categorized as such. The units operations and processes in Waste-water treatment are summarized as follows (Economic and Social Commission for Western Asia (ESCWA), 2003):

Physical unit operations

- Screening
- Comminution
- Flow equalization
- Sedimentation
- Flotation
- Granular-medium filtration

Chemical unit operations

- Chemical precipitation
- Adsorption
- Disinfection
- Dechlorination

- Other chemical applications

Biological unit operations

- Activated sludge process
- Aerated lagoon
- Trickling filters
- Rotating biological contactors
- Pond stabilization
- Anaerobic digestion

3.2 Levels of wastewater treatment

There are three broad levels of treatment: primary, secondary and tertiary. Sometimes, preliminary treatment precedes primary treatment.

Preliminary treatment: removes coarse suspended and grits. These can be removed by screening, and grit chambers respectively. This enhances the operation and maintenance of subsequent treatment units. Flow measurement devices, often standing-wave flumes, are necessary at this treatment stage (FAO, 2006).

Primary treatment removes settleable organic and inorganic solids by sedimentation and floating materials (scum) by skimming. Up to 50% of BOD5, 70% of suspended solids and 65% of grease and oil can be removed at this stage. Some organic nitrogen, organic phosphorus, and heavy metals are also removed. Colloidal and dissolved constituents are however not removed at this stage. The effluent from primary sedimentation units is referred to as primary effluent (FAO, 2006).

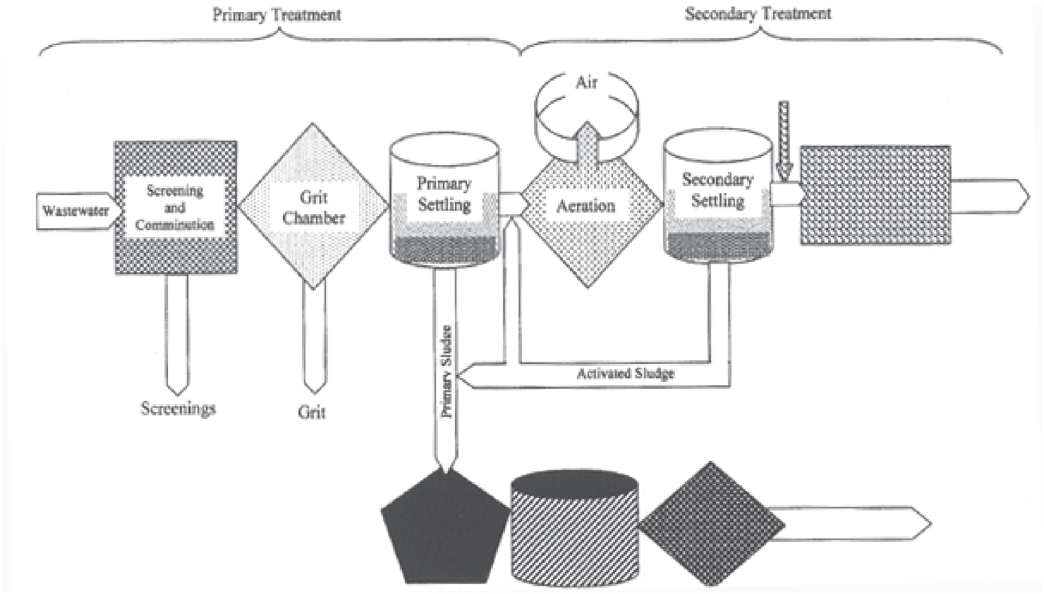
Secondary treatment is the further treatment of primary effluent to remove residual organics and suspended solids. Also biodegradable dissolved and colloidal organic matter is removed using aerobic biological treatment processes. The removal of organic matter is when nitrogen compounds and phosphorus compounds and pathogenic microorganisms are removed. The treatment can be done mechanically like in trickling filters, activated sludge methods rotating biological contactors (RBC) or non-mechanically like in anaerobic treatment, oxidation ditches, stabilization ponds etc.

Tertiary treatment or advance treatment is employed when specific wastewater constituents which cannot be removed by secondary treatment must be removed. Advance treatment removes significant amounts of nitrogen, phosphorus, heavy metals, biodegradable organics, bacteria and viruses. Two methods can be used effectively to filter secondary effluent—traditional sand (or similar media) filter and the newer membrane materials. Some filters have been improved, and both filters and membranes also remove helminths. The latest method is disk filtration which utilizes large disks of cloth media attached to rotating drums for filtration (FAO, 2006).

At this stage, disinfection by the injection of Chlorine, Ozone and Ultra Violet (UV) irradiation can be done to make water meet current international standards for agricultural and urban re-use.

4. Methods of wastewater treatment

There are conventional and non-conventional wastewater treatment methods which have been proven and found to be efficient in the treatment of wastewater. Conventional methods compared to non-conventional wastewater treatment methods has a relatively high



Source: NPTEL (accessed 2010)

Fig. 2. Typical Wastewater Treatment Plant

level of automation. Usually have pumping and power requirements. They require skilled labour for operation and maintenance of the system

4.1 Conventional methods

Examples of conventional wastewater treatment methods include activated sludge, trickling filter, rotating biological contactor methods. Trickling filters and Rotating Biological Contactors are temperature sensitive, remove less BOD, and trickling filters cost more to build than activated sludge systems. Activated sludge systems are much more expensive to operate because energy is needed to run pumps and blowers (National Programme on Technology Enhanced Learning (NPTEL), 2010).

These methods are discussed in detail in the subsequent sections.

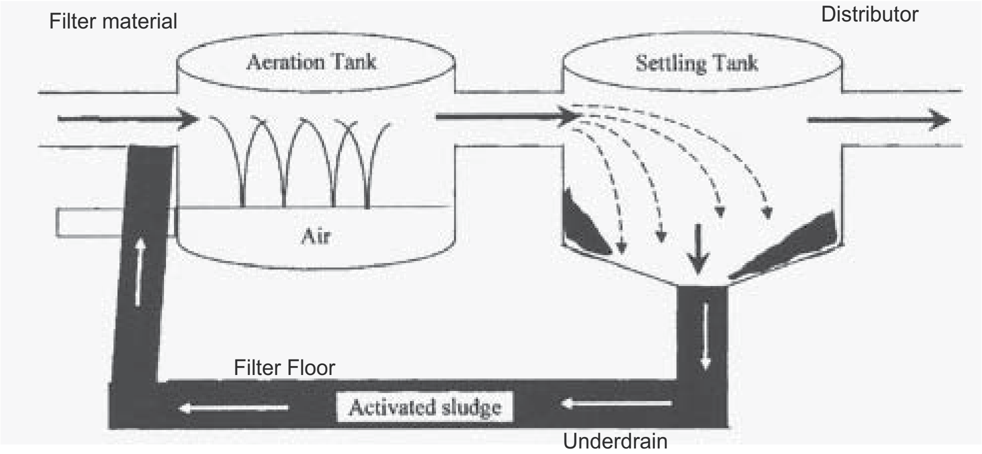
4.1.1 Activated sludge

Activated sludge refers to biological treatment processes that use a suspended growth of organisms to remove BOD and suspended solids. It is based on the principle that intense wastewater aeration to forms flocs of bacteria (activated sludge), which degrade organic matter and be separated by sedimentation. The system consists of aeration and settling tanks with other appurtenances such as return and waste pumps, mixers and blowers for aeration and a flow measurement device. To maintain the concentration of active bacteria in the tank, part of the activated sludge is recycled.

Primary effluent (or plant influent) is mixed with return activated sludge to form mixed liquor which is aerated for a specified length of time. By aerating the system, activated sludge organisms use the available organic matter as food, thereby, producing stable solids and more organisms. The suspended solids produced by the process and the additional organisms become part of the activated sludge. The solids are then separated from the wastewater in the settling tank and are returned to the influent of the aeration tank (return activated sludge). Periodically the excess solids and organisms are removed from the system (waste activated sludge) to enhance the performance of the system.

Factors such as temperature, return rates, amount of oxygen available, amount of organic matter available, pH, waste rates, aeration time, and wastewater toxicity affect the performance of an activated sludge treatment system. A balance therefore must be maintained between the amount of food (organic matter), organisms (activated sludge) and dissolved oxygen (NPTEL, 2010).

Activated Sludge systems are requires less space compared to trickling filter and has high effluent quality. The disadvantage is that BOD is higher at one end of the tank than the other the microorganisms will be physiologically more active at that end than the other unless a complet mixing activated sludge system process is used. Presently there are 11 activated sludge plants in Ghana, mainly installed by the large hotels (Obuobie, et al., 2006).



Source: Mountain Empire College, 2010

Fig. 3. An activated Sludge System

4.1.2 Trickling filter:

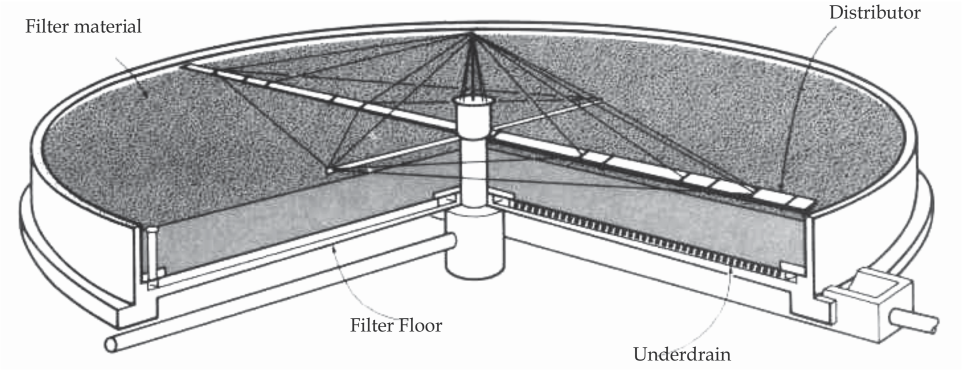
It is a growth process in which microorganisms responsible for treatment are attached to an inert packing material. It is made up of a round tank filled with a carrier material (volcanic rock, gravel or synthetic material). Wastewater is supplied from above and trickles through filter media allowing organic material in the wastewater to be adsorbed by a population of microorganisms (aerobic, anaerobic, and facultative bacteria; fungi; algae; and protozoa) attached to the medium as a biological film or slime layer (approximately 0.1 to 0.2 mm thick).

Degradation of organic material by the aerobic microorganisms in the outer part of the slime layer occurs. As the layer thickens through microbial growth, oxygen cannot penetrate the medium face, and anaerobic organisms develop. The biological film continues to grow to such a point that microorganisms near the surface cannot cling to the medium, and a portion of the slime layer falls off the filter. This process is known as sloughing. The sloughed solids are picked up by the underdrain system and transported to a clarifier for removal from the wastewater (US EPA, 2000).

Trickling filters are efficient in that effluent quality in terms of BOD and suspended solids removal is high. Its operational costs are relatively low due to low electricity requirements. The process is simpler compared to activated sludge process or some package treatment plants. Its operation and maintenance requirements is however high due to the use of electrical power. Skilled labour is required to keep the trickling filter running trouble-free: e.g. prevent clogging, ensure adequate flushing, control filter flies. It is suitable for some relatively wealthy, densely populated areas which have a sewerage system and centralized wastewater treatment; also suitable for greywater treatment.

It also requires more space compared to some other technologies and has potential for odour and filter flies (NPTEL, 2010).

This method has been widely used in Ghana. There are 14 trickling filter plants in Accra though they have broken down.



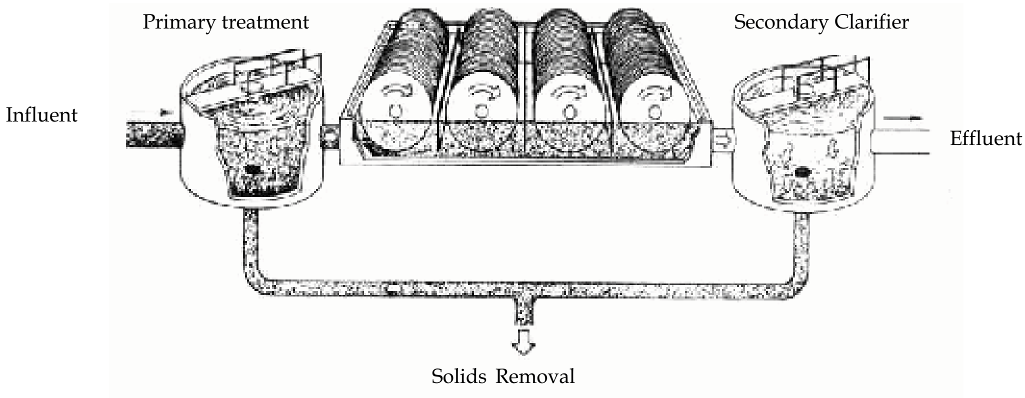
Source: ESCWA, 2003

Fig. 4. Cross section of a trickling filter

4.1.3 Rotating biological contactors

Rotating biological contactors (RBCs) consist of vertically arranged, plastic media on a horizontal, rotating shaft. The plastics range from 2 – 4 m in diameter and up to 10 mm thick (Peavy, Rowe ad Tchobanoglous, 1985). The biomass-coated media are alternately exposed to wastewater and atmospheric oxygen as the shaft slowly rotates at 1–1.5 rpm (necessary to provide hydraulic shear for sloughing and to maintain turbulence to keep solid in suspension), with about 40% of the media submerged. High surface area allows a large, stable biomass population to develop, with excess growth continuously and automatically shed and removed in a downstream clarifier. Thichness of biofilm may reach 2 – 4 mm depending on the strength of wastewater and the rotational speed of the disk.

RBC systems are relatively new, though it appeared to be best suited to treat municipal wastewater (Peavy, Rowe ad Tchobanoglous, 1985), they have been installed in many petroleum facilities because of their ability to quickly recover from upset conditions (Schultz, 2005). The RBC system is easily expandable should the need arise, and RBCs are also very easy to enclose should volatile organic content containment become necessary. RBCs have relatively low power requirements and can even be powered by compressed air which can also aerate the system. They follow simple operating procedures and thus require a moderately skilled labour. RBCs are however capital intensive to install and sensitive to temperature.

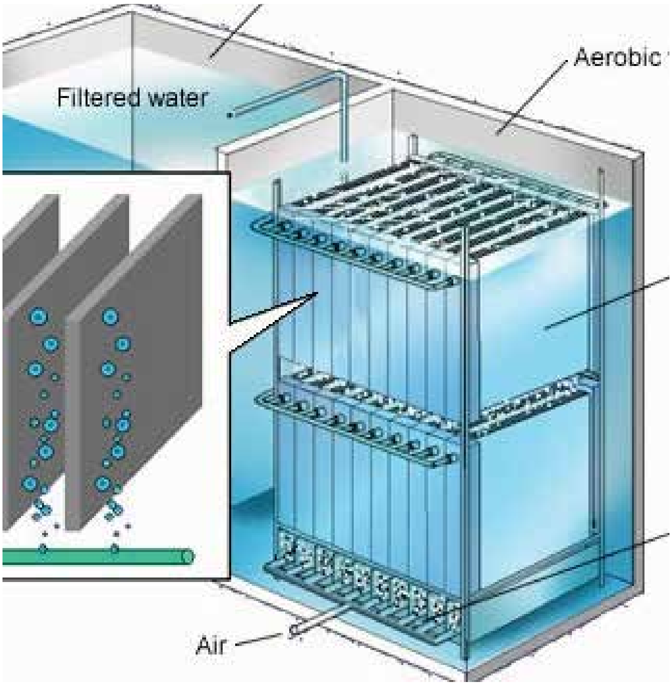


Source: ESCWA, 2003

Fig. 5. Rotating Biological Contactors

4.1.4 Membrane bioreactors

This method performs more than just one treatment step. Membrane bioreactor (MBR) systems are unique processes, which combine anoxic- and aerobic-biological treatment with an integrated membrane system that can be used with most suspended-growth, biological wastewater-treatment systems.



Source: Google Images

Fig. 6. Membrane Bioreator

Wastewater is screened before entering the biological treatment tank. Aeration within the aerobic-reactor zone provides oxygen for biological respiration and maintains solids in suspension. MBR relies on submerged membranes to retain active biomass in the process. This allows the biological process to operate at longer than normal sludge ages (typically 20-100 days for a MBR) and to increase mixed-liquor, suspended-solids (MLSS) concentrations (typically 8,000-15,000 mg/l) for more effective removal of pollutants. High MLSS concentrations reduce biological-volume requirements and the associated space needed to only 20–30% of conventional biological processes.

MBRs cover a small land area as it eliminates the need for secondary clarifiers, which equates to a huge savings in both footprint and concrete costs. They can operate at higher biomass concentrations (MLSS) than conventional treatment processes. Facility can be expanded by simply adding more membranes to existing basins without expanding land cover. For reuse quality, it does not require tertiary treatment, polymer addition, or any further treatment processes to meet standards. This reduction in the number of unit processes further improves system reliability and reduces operation activities (TEC, 2010). The generally high effluent quality reduces the burden on disinfection in the treatment process.

4.2 Non-conventional methods

These are low-cost, low-technology, less sophisticated in operation and maintenance biological treatment systems for municipal wastewater. Although these systems are land intensive by comparison with the conventional high-rate biological processes, they are often more effective in removing pathogens and do so reliably and continuously if system is properly designed and not overloaded (FAO, 2006). Some of the non-conventional methods include stabilization ponds, constructed wetlands, oxidation ditch, soil aquifer treatment.

4.2.1 Waste stabilization ponds

Waste Stabilization Ponds are man-made, shallow basins which comprise of a single series or several series of anaerobic, facultative or maturation ponds. This is a low-technology treatment process with 4 or 5 ponds of different depths with different biological activities. Treatment of the wastewater occurs as constituents are removed by sedimentation or transformed by biological and chemical processes (National Academy, 2005).The anaerobic ponds are mainly designed for the settling and removal of suspended solids as well as the breakdown of some organic matter (BOD_5). In facultative ponds, organic matter is further broken down to carbon dioxide, nitrogen and phosphorous by using oxygen produced by algae in the pond. Maturation ponds usually remove nutrients and pathogenic micro-organisms, thus primary treatment occurs in anaerobic ponds while secondary and tertiary treatment occurs in facultative and maturation ponds respectively (Awuah, 2002).

Anaerobic ponds are usually between 2-5 m deep and receive high organic loads equivalent to 100g BOD_5 and m^3/d leading to anaerobic conditions throughout the pond (Mara *et al.,* 1992). If properly designed, anaerobic ponds can remove 60% of BOD_5 at 200 C.

Facultative ponds are 1-2 m deep and usually receive the effluent from an anaerobic pond. In some designs, they receive raw wastewater acting as primary facultative pond. In facultative ponds organic loads are lower and allows for algal growth which accounts for the dark green colour of wastewater. Algae and aerobic bacteria generate oxygen which breaks down BOD_5. Good wind velocity generates mixing of wastewater in ponds thus leading to uniform mixing of BOD_5, oxygen, bacteria and algae which better stabilizes waste.

Maturation ponds are usually shallow ponds of about 1.0-1.5 m deep allowing aerobic conditions in for the treatment of facultative pond effluents. Further reduction of organic matter, nutrients and pathogenic microorganisms occurs here. Algal population in maturation ponds is more diverse and removal of nitrogen and ammonia is more prominent.

In Ghana so far, stabilization ponds have worked very well due to the convenient climatic conditions. It usually flows under gravity from one pond to the other and mostly does not require any pumping. It is less energy dependent thus plant activities cannot be interrupted due to power cuts. Its disadvantages however include odour problems and it requires a large area of land to function properly. Presently there are 21 stabilisation ponds in Ghana mainly in Accra and Kumasi. Some of them like the Tema Community 3, Achimota, have been closed Various combinations and arrangement of ponds are possible. The figure below shows some possible combinations.

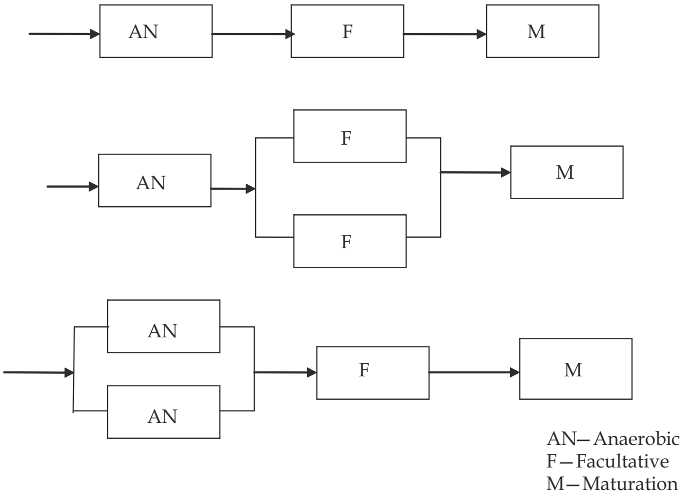
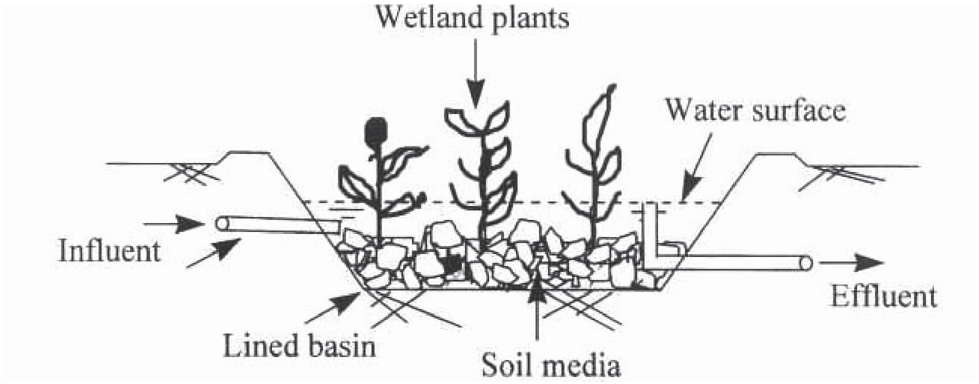


Fig. 7. Various Arrangement of Waste Stabilisation Ponds

4.2.2 Constructed wetlands

Constructed Wetlands (CW's) are planned systems which are designed and constructed to employ wetland vegetation to assist in treating wastewater in a more controlled environment than occurs in natural wetlands (Kayombo et al., 2000). They are an eco-friendly and a suitable alternative for secondary and tertiary treatment of municipal and industrial wastewater. They are suitable for the removal of organic materials, suspended solids, nutrients, pathogens, heavy metals and toxic pollutants. They are not ideal for the treatment of raw sewage, pre-treatment of industrial wastewater to maintain the biological balance of the wetland ecosystem.

There are two types of CW's namely Free Water Surface (FWS) and Subsurface Flow (SSF) systems. As the name suggests, with FWS, water flows above the ground and plants are rooted in the sediment layer below the water column. With SSF, water flows through a porous media such as gravels in which the plants are rooted. From a public health perspective, SSF should be used in primary treatment of wastewater because there is no direct contact of wastewater with atmosphere.

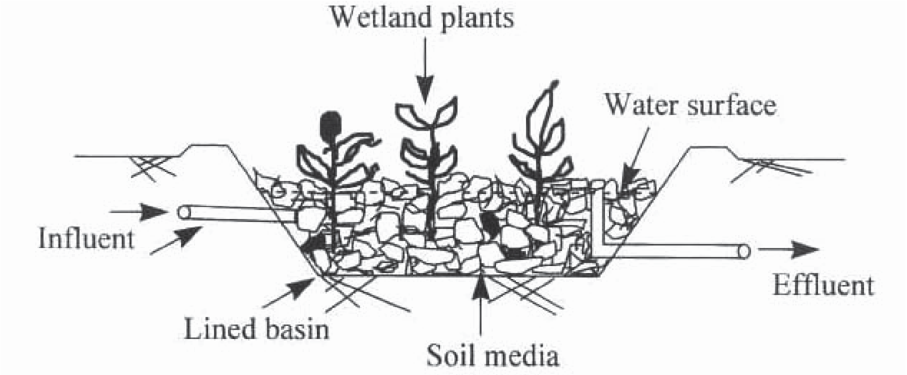


Source: ESCWA, 2003

Fig. 8. Free Water Surface System

The SSF is mostly anoxic or anaerobic as oxygen supplied by the roots of plants is used up in biofilm growth and as such does not reach the water colomn. The flow of water in SSF can be horizontal or vertical (Kayombo *et al.*, 2000). FWS are suitable for treating secondary and tertiary effluents and also providing habitat due to aerobic conditions at and near the surface of the water. There condition at the bottom sediment is however anoxic.

Wetlands plants or macrophytes utilized in CW's include Cattails (*Typha latifolia sp), Scirpus* (Bulrus), *Lemna* (duckweed), *Eichornia crassipes* (water hyacinth), *Pistia stratiotes* (water lettuce) *Hydrocotyle* spp. (pennywort), *Phragmites* (reed) have been known and used in constructed wetlands.



Source: ESCWA, 2003

Fig. 9. Sub-surface flow system

CW's are relatively cheaper to construct operate and easy to maintain. This is an important decision variable for developing countries. In Egypt, according to Hendy (2006), between 2000 and 2004, a 60 acre artificial wetland constructed cost 25% the cost of conventional sewage treatment plant.

They provide effective and reliable treatment of wastewater and are tolerant to fluctuating hydrologic and contaminant loading rates. With the example in Egypt, $9 million (US) was spent to treat an initial volume of 25,000 metric tons per day. After a year of use, it was determined that the wetland was capable of treating 40,000 metric tons per day (Hendy, 2006). Also a study conducted by Ratnapriya *et al.,* (2009) revealed over 60% removal of BOD5, COD, nitrogen among others.

CW's also provide indirect benefits such as enjoying the scenic views of green spaces, encouraging wildlife habitats and providing recreational and educational centres. Again, in Egypt, the fishing industry is expanding since the wastewater was no longer being discharged directly into the waterways, the local fisheries improved. According to Hendy (2006), nitrates and heavy metals were filtered out, leaving the fish healthier, larger and in abundant quantity. This indirectly led to poverty reduction.

They however have some disadvantages such as land requirements, its design and operation criteria is presently imprecise. CW's are biologically and hydrologically complex and its process dynamics are not completely understood. Sometimes there are cost implications of gravels fills and site grading during construction (Kayombo *et al.,* 2000). It must be emphasized that if properly designed, constructed wetlands should not breed pests and mosquitoes.

In Ghana, there are not many CW's. There is presently a pilot SSF donor CW in Tema. This plant is not entirely low-cost as it was designed with some energy dependent units.

4.2.3 Oxidation ditches

An oxidation ditch is a modified activated sludge biological treatment process that utilizes hydraulic retention time of 24 - 48 hours, and a sludge age of 12 - 20 days. to remove biodegradable organics. Oxidation ditches are typically complete mix systems, but can be modified. Typical oxidation ditch treatment systems consist of a single or multichannel configuration within a ring, or oval. Preliminary treatment, such as bar screens and grit removal, normally precedes the oxidation ditch. Primary settling prior to an oxidation ditch is sometimes practiced and tertiary filters may be required after clarification, depending on the effluent requirements. Disinfection is required and reaeration may be necessary prior to final discharge. Horizontally or vertically mounted aerators provide circulation, oxygen transfer, and aeration in the ditch. Flow to the oxidation ditch is aerated and mixed with return sludge from a secondary clarifier. The mixing process entrains oxygen into the mixed liquor to foster microbial growth and the motive velocity ensures contact of microorganisms with the influent. Aeration increases dissolved oxygen concentration but decreases as biomass takes up oxygen during mixing in the ditch. Solids also remain in suspension during circulation (USEPA, 2000).

They require more power than waste stabilization ponds less land, and are easier to control than processes such as activated sludge process. A typical process flow diagram of treatment plant using an oxidation ditch is shown in Figure 10.

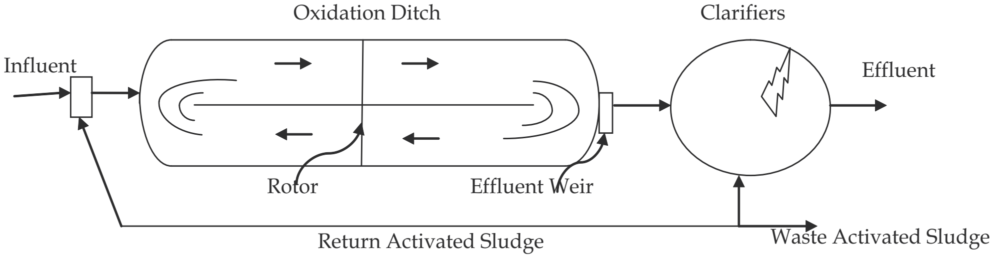
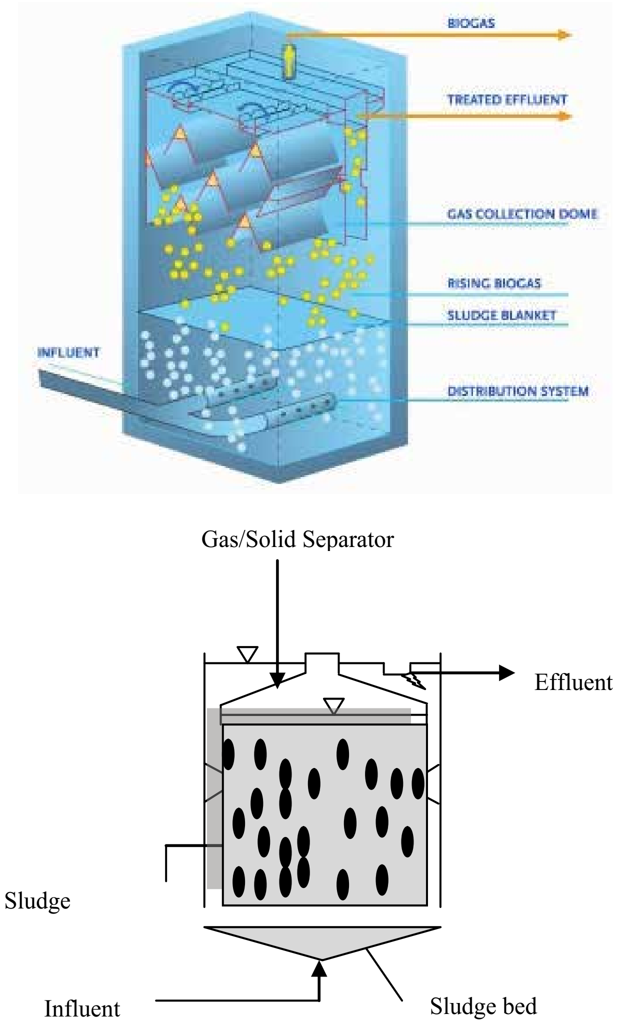


Fig. 10. Oxidation Ditch

4.2.4 Upflow anaerobic sludge blanket (UASB)

Upflow anaerobic sludge blanket is an anaerobic process using blanket of bacteria (see Figure 11) to absorb polluting load. It is a form of anaerobic digester which forms a blanket of granular sludge which suspends in the tank. Wastewater flows upwards through the blanket and is processed (degraded) by the anaerobic microorganisms. The upward flow combined with the settling action of gravity suspends the blanket with the aid of flocculants. Small sludge granules begin to form whose surface area is covered in agregations of bacteria. In the absence of any support matrix, the flow conditions create a selective environment in which only those microorganisms, capable of attaching to each other, survive and proliferate.



Source: Google Images

Fig. 11. Upflow Anaerobic Sludge Blanket

Eventually the aggregates form into dense compact biofilms referred to as granules. The UASB reactor works best when desirable micro-organisms are retained as highly active and fast settling granules. In the UASB reactor, when high solids retention time is met, separation of gas, sludge solids from the liquid occurs. The special Gas-Solid-Liquid Separators in the reactor enable collection of biogas and recycle of anaerobic biomass. Biogas contains 50 to 80% methane.

UASB is suitable for the primary treatment of high-COD mainly soluble industrial effluents. It can also be used for the treatment of wastewater effluents of low and medium strength. It is suited to hot climates Low energy requirement, less operation and maintenance, lower skill requirement for operation, less sludge production, resource recovery through biogas generation and stabilized waste as manure. UASBs however have relatively poor effluent quality than processes such as activated sludge process (Tare and Nema, 2010).

The technology however, needs constant monitoring to ensure that the sludge blanket is maintained, and not washed out. The heat produced as a by-product of electricity generation can be reused to heat the digestion tanks.

4.2.5 Soil aquifer treatment

Soil matrix has quite a high capacity for treatment of normal domestic sewage, as long as capacity is not exceeded. Partially-treated sewage effluent is allowed to infiltrate in controlled conditions to the soil. The unsaturated or "vadose" zone then acts as a natural filter and can remove essentially all suspended solids, biodegradable materials, bacteria, viruses, and other microorganisms. Significant reductions in nitrogen, phosphorus, and heavy metals concentrations can also be achieved. After the sewage, treated in passage through the vadose zone, has reached the groundwater it is usually allowed to flow some distance through the aquifer for further purification before it is collected through the aquifer.

Soil-aquifer treatment is a low-technology, advanced wastewater treatment system. It also has an aesthetic advantage over conventionally treated sewage since effluent from an SAT systems is clear and odour-free and it is viewed as groundwater either than effluent. Discharge effluent should travel sufficient distance through the system and residence times should be long enough, to produce effluent of desired quality (FAO, 2006).

4.3 Faecal sludge treatment and disposal

Sewage sludge contains organic and inorganic solids that were found in the raw wastewater. Sludge from primary and secondary clarifier as well as from secondary biological treatment need to be treated. The generated sludge is usually in the form of a liquid or semisolid, containing 0.25 to 12 per cent solids by weight, depending on the treatment operations and processes used. Sludge is treated by means of a variety of processes that can be used in various combinations. Thickening, conditioning, dewatering and drying are primarily used to remove moisture from sludge, while digestion, composting, incineration, wet-air oxidation and vertical tube reactors are used to treat or stabilize the organic material in the sludge (ESCWA, 2003).

Thickening: Thickening is done to increase the solids content of sludge by the reduction of the liquid content. An increase in solids content from 3 to about 6 per cent can decrease total sludge volume significantly by 50 per cent. Sludge thickening methods are usually physical in nature: they include gravity settling, flotation, centrifugation and gravity belts.

Stabilization: Sludge stabilization is aimed at reducing the pathogen content, eliminate offensive odours, and reduce or eliminate the potential for putrefaction. Some methods used for sludge stabilization include lime stabilization, heat treatment, anaerobic digestion, aerobic digestion and composting (ESCWA, 2003).

5. Wastewater reuse in agriculture

Irrigation with wastewater is both disposal and utilization and indeed is an effective form of wastewater disposal (as in slow-rate land treatment). However, some degree of treatment must normally be provided to raw municipal wastewater before it can be used for agricultural or landscape irrigation or for aquaculture.

In many industrialized countries, primary treatment is the minimum level of preapplication treatment required for wastewater irrigation. It may be considered sufficient treatment if the wastewater is used to irrigate crops that are not consumed by humans or to irrigate orchards, vineyards, and some processed food crops (FAO, 2006).

Nutrients in municipal wastewater and treated effluents are a particular advantage as supplemental fertilizers. Success in using treated wastewater for crop production will largely depend on adopting appropriate strategies aimed at optimizing crop yields and quality, maintaining soil productivity and safeguarding the environment. Several alternatives are available and a combination of these alternatives will offer an optimum solution for a given set of conditions. The user should have prior information on effluent supply and its quality. Wastewater effluent can be blended with conventional water or solely used. Heavy metal concentrations in streams used for irrigation in and around urban centres such as Accra and Kumasi have been sometimes found to be beyond recommended levels for irrigation purposed and should therefore may pose a health concern.

Countries must develop standards in congruence with the WHO guidelines and enforce it.

6. Industrial wastewater treatment

In general, the type of plant to be installed depends on the characteristics of the wastewater produced from that industry. The basic principle according to Kamala and Kanth Rao (1989) however is waste prevention by good housekeeping practices that will ultimately result in volume reduction and strength reduction. Industrial wastewater is treated the same way as domestic or municipal sewage—preliminary, primary, secondary and advanced treatment levels. Most of the treatment methods discussed is also applicable. There could however be peculiarities with different industrial depending on their major contaminant e.g. heavy metals, dye, etc.

Industrial wastewater in Ghana is generated from breweries, distilleries, textile, chemical & pharmaceuticals and institutions and hotels which are mainly situated in Accra and Tema. In the Western and middle belt of Ghana, mining activities are predominant and the major polluter of our rivers. EPA-Ghana grants permits to industries and requires industries to install or build an in-house waste treatment plant. EPA-Ghana takes samples quarterly from these industrial wastewater plants for testing in their own laboratories for monitoring purposes. Most of those who have permit have treatment plants though not all of them are functioning properly.

In recent years, there has been a growth of small-scale industries in the fruits and food processing industries in the Tema, light industrial area which do not have the resources to build treatment plant. Most of these small-scale industries empty their wastewater into nearby drains without treatment. In Kumasi, the principal generators of industrial wastewater in Kumasi are the two breweries, a soft drink bottling plant and an Abattoir.

7. Status of wastewater treatment plants in Ghana

The use of on-site treatment systems is quite extensive. Individual and community/residential based septic tanks are the most preferred. Septic tanks only partially treat sewage, and the effluent is still rich in organic material. The septic tank has to be emptied from time to time and the disposal of the septic sludge causes severe public health and environmental particularly in urban areas.

Major wastewater treatment methods found in Ghana includes stabilisation ponds, trickling filters and activated sludge plants. According to a recent survey, there are 46 wastewater treatment plants in Ghana. More than half of all treatment plants in Ghana are in the Greater Accra region, mainly in the capital city of Accra and port city of Tema. Brong Ahafo and Upper West regions have no treatment plants at all. The stabilization pond method is the most extensively used with almost all faecal sludge and large-capacity sewage treatment plants using the method. Most trickling filters and activated sludge plants recorded have a low capacity and belong to private enterprises like larger hotels.

Only about 10 of the treatment plants are operational (Obuobie *et al.*, 2006) and it is not clear if these plants meet the EPA effluent guidelines. This can be attributed to the fact that the conventional methods are energy dependent and also when the mechanical parts become faulty, the part has to be imported making it too expensive to maintain. Low-cost, low-technology methods are however manageable.

8. Challenges of wastewater management

Wastewater management though not technically difficult can sometimes be faced with socio-economic challenges. A few of the challenges are discussed below.

8.1 Infrastructure

Most often than not, wastewater infrastructure are not the priority of most politicians and therefore very little investment are made. It is however important to consider wastewater infrastructure as equally important as water treatment plant because almost all the water produced ends up as wastewater.

8.2 Pollution of water sources

Effects of wastewater effluent on receiving water quality is enormous, it changes the aquatic environment thus interrupts with the aquatic ecosystem. The food we eat contains carbonaceous matter, nutrients, trace elements and salts and are contained in urine and faeces (black water).

Medications (drugs), chemicals and in recent times hormones (contraceptives) are also discharged into the wastewater treatment plant. Discharge guidelines must be strictly adhered to. This will ensure sustainability of water sources for posterity.

The precautionary and the polluter-pays principles which prevent or reduce pollution to the wastewater have proven to be very efficient in the industrialized countries and should be adapted in developing countries as well.

8.3 Choice of appropriate technology

Because the economy of most developing countries is donor driven, funds for wastewater plants are mainly from donors. For this reason, they tend to propose the technology which should be adopted. For this reason, when the beneficiaries, take over the facility, its management of the operations and maintenance of parts become quite challenging as the technical expertise, power requirements etc are not sustainable.

8.4 Sludge production

Treatment of wastewater results in the production of sewage sludge. There must be a reliable disposal method. If it must be used in agriculture, then the risks involved must be taken into consideration. Due to the presence of heavy metals in wastewater, it is sometimes feared that agricultural use may lead to accumulation of heavy metals in soils thereby contaminating of yields.

8.5 Reuse

Effluents which meet discharge standards could be used for agricultural purposes such as aquaculture or for irrigation of farmlands. The challenge however is that if wastewater treatment plants are not managed and continuously monitored to ensure good effluent quality, reuse becomes risky.

9. Conclusion

Wastewater is and will always be with us because we cannot survive without water. When water supplied is used for the numerous human activities, it becomes contaminated or its characteristics is changed and therefore become wastewater. Wastewater can and must be treated to ensure a safe environment and foster public health. There are conventional and non-conventional methods of wastewater treatment and the choice of a particular method should be based on factors such as characteristics of wastewater whether it from a municipality or industry (chemical, textile, pharmaceutical etc.), technical expertise for operation and maintenance, cost implications, power requirements among others.

In most developing countries like Ghana, low-cost, low-technology methods such as waste stabilization ponds have been successful whilst conventional methods like trickling filters and activated sludge systems have broken down. Effluent which meets set discharge standards can be appropriately used for aquaculture and also irrigation. Though there are a few challenges in waste water management, they can be surmounted if attention and the necessary financial support is given to it.

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Sewage Sludge Disposal and Applications: Self-heating and Spontaneous Combustion of Compost Piles - Trace Metals Leaching in Volcanic Soils After Sewage Sludge Disposal

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1. Introduction

1.1 Introduction

Municipal solid waste landfills often develop scenarios of self heating, causing negative environmental impacts by odors, gas generation and smoke production. Self-ignition and resulting fires at landfills have been undesirable outcomes in compost piles worldwide. Field, laboratory and numerical studies have been considered trying to reproduce and understand the conditions under which self-heating and combustion may take place. Inside a compost pile built from solids obtained after municipal wastewater treatment, oxygen, methane, carbon monoxide and carbon dioxide concentrations and temperature change with time and in depth. Electric conductivity and pH show only slight changes. In field piles temperature increased with time, reaching a maximum of about 90°C. While no spontaneous combustion was observed after six months in field experiments, in laboratory studies carried out in a closed bottom cylinder, self-ignition was observed and a maximum temperature of about 400°C was reached. Spontaneous combustion in compost piles is a chemical and biological process. Initially, the metabolism that allows microorganism growth causes temperature increase, but simultaneous oxidation of the organic matter also reinforces self-ignition at a lower value. When the temperature within the compost pile rises to about 87°C, self-ignition follows mainly due to cellulose oxidation. Therefore, the conditions under which biomass increases must be studied. An important factor in the development of internal changes within the compost pile comes from atmospheric boundary conditions. General convection, radiation, rain rate and atmospheric humidity must be included as boundary atmospheric field conditions on the lateral and top surfaces.

Local thermal equilibrium is assumed, which is a common assumption for porous media and packed particle beds, hence effective properties can describe the porous media. Coupled two-dimensional heat and oxygen diffusion in a compost pile of sewage sludge obtained from domestic wastewater treatment can be described using a transient, nonlinear mathematical model that includes the volumetric heat generation caused by the action of aerobic bacteria and by the oxidation of cellulose in a porous medium. Numerical simulations with the finite volume method allow the prediction of the pile's shape and size effects on the heat generated and on oxygen consumption. Transient temperature and oxygen concentration distributions within compost piles depend on their geometry. Heat (temperature) and mass transfer (oxygen) results indicate that pile height has an important effect on the heating. The volumetric heat generation caused by the action of aerobic bacteria and by the oxidation of cellulose in a porous medium must be incorporated as a function of the mean moisture by an essentially thermodynamic source term. Inside a compost pile made of solid residues obtained after municipal wastewater treatment, temperature changes, oxygen content and moisture can be measured in time and depth. The Richards equation is a standard, frequently used approach for modeling and describing water flow in variably saturated porous media such as soils and compost piles. The nonlinear mathematical model considers the Richards equation and a second mass transport equation for water content, and therefore the numerical simulations can describe the internal changes in heat, oxygen and moisture observed under field conditions. Therefore a model with three diffusion equations must be used to quantify the effects of moisture on temperature and oxygen concentration.

1.2 Materials and methods. Experimental conditions

For field and laboratory studies sewage sludge from "El Trebal", a domestic wastewater treatment plant located close to the city of Santiago, Chile, was used, where about 4 m^3/s of wastewater are treated. In the field, four sewage sludge piles 1.5 and 2.5 m high, 6.2-7 m wide, and 8.5 m long, with a 3-D trapezoidal shape and a bulk density of 0.75 ton/m^3 were prepared. Temperatures were measured using type K thermocouples (Ni-Cr) connected to an LA/AI-A8 Cole Parmer data acquisition system, and they were recorded every 5 min, during 80 to 120 hours. Measurements of humidity, O_2, CH_4, CO_2, and CO were made twice a week; pH, electric conductivity, and organic carbon were determined once a week. Water content was determined from the weight lost after heating 10 g of sample at 105 °C for 24 hours, and electrical conductivity and pH were determined in a 1:2.5 solid:water ratio extract. Organic carbon content (expressed as %OC), was determined by a wet oxidation method (Walkley & Black, 1934) where the sample reacts with a mixture of 1 N $K_2Cr_2O_7$ and concentrated H_2SO_4.

A Dräger Miniwarn equipment, equipped with an IR catalytic sensor for CH_4 and electrochemical sensors for O_2, CO_2 and CO, was used considering a 1.5 L/min mass flow.

Field experiments were carried out for 20 weeks, a period during which wind speed and direction, relative humidity of air, and precipitation were also recorded.

In the laboratory a stainless steel cylinder 20 cm in diameter, 20 cm deep, and 2 mm wall thickness was used. The cylinder was externally insulated, with openings at depths of 0.0, 1.0, 2.0, 2.5, 3.0, 7.0, 10.0 and 15.0 cm for thermocouples location. An adjustable electric heater was incorporated 2.5 cm over the cylinder, and experiments were carried out at different external temperatures. Sewage sludge was placed in the cylinder at the same bulk density observed in the field.

1.3 Mathematical modeling and numerical simulation of compost pile. Self-heating in compost pile, differential equations for temperature and oxygen concentration.

Temperature and oxygen concentration are the important dependent variables for describing heat and mass transfer processes inside compost piles. Basic equations to build appropriate mathematical models, numerical solutions procedures, and the results obtained are presented in this section.

The mathematical model considers transient coupled two-dimensional heat and oxygen diffusion in porous media (Sidhu et al., 2007). The pile bottom is assumed to be adiabatic, as shown in Figure 1.

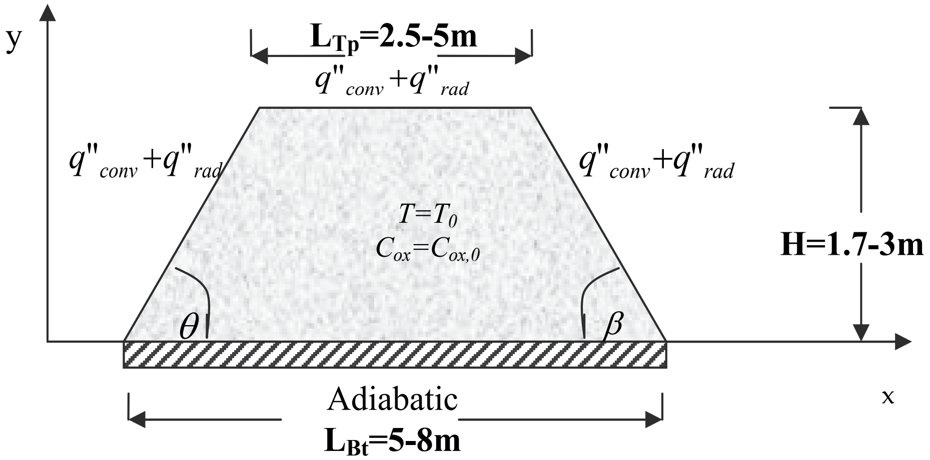


Fig. 1. Physical situation of the compost pile

Cellulosic oxidation and micro organism activity inside the pile are incorporated in the model by volumetric heat generation. For simplicity, local thermal equilibrium is assumed, which is a common assumption for porous medium and packed particle beds (Nield & Bejan, 1992).

$$\frac{\partial(\rho C)_{eff}T}{\partial t} = \nabla k_{eff}\nabla T + Q_c(1-\varepsilon)A_c\rho_c C_{ox}\exp\left(\frac{-E_C}{RT}\right) + Q_b(1-\varepsilon)\rho_b\rho_c\left(\frac{A_1\exp\left(\frac{-E_1}{RT}\right)}{1+A_2\exp\left(\frac{-E_2}{RT}\right)}\right) \tag{1}$$

$$\varepsilon\frac{\partial C_{ox}}{\partial t} = \nabla D_{eff}\nabla C_{ox} - (1-\varepsilon)A_c\rho_c C_{ox}\exp\left[\frac{-E_c}{RT}\right] \tag{2}$$

In equations (1) and (2) A_c is the pre-exponential factor for the oxidation of the cellulose and E_c, E_1, E_2, are the activation energy for cellulose, biomass growth and inhibition of biomass growth, respectively. Heat and mass transfer properties in the porous media are defined in terms of the pile's porosity ε,

$$k_{eff} = \varepsilon k_{air} + (1-\varepsilon)k_c \tag{3}$$

$$(\rho C)_{eff} = \varepsilon\rho_{air}C_{air} + (1-\varepsilon)\rho_c C_c \tag{4}$$

$$D_{eff} = \varepsilon D_{air,c} \tag{5}$$

where k_{eff} and D_{eff} are the effective properties which are considered independent of temperature and concentration, and C_c is the specific heat capacity of cellulose. The heat produced by the oxidation of the cellulosic material is represented by the second term on the right-hand side of the Equation (1). This term is expressed as a function of oxygen concentration and is responsible for the self-heating. The last term in Equation (1) represents heat generated due to biological activity within the pile, caused by microorganism growth. Oxygen content variation is included in Equation (2). Initially, oxygen content in the pile has the same concentration as in the external air. Micro-organisms growth followed by organic matter oxidation produces the oxygen depletion. These assumptions are incorporated in the second term on the right-hand side of the Equation (2).

Details in the formulation of the term representing the heat generated by the biomass have been given by Chen and Mitchell (Chen & Mitchell, 1996) and some parameter values used in the mathematical model may be obtained from the literature (Sidhu et al., 2007; Escudey et al., 2008; Moraga et al., 2009).

1.4 Temperature and oxygen concentration, initial and boundary conditions

Initial temperature and oxygen and moisture distribution within the pile are assumed starting from the corresponding first experimental values available:

$$T(x,y,0) = T_0 \, ; \qquad C_{ox}(x,y,0) = C_{ox,0} \tag{6}$$

Adiabatic and impermeable boundary conditions are considered at the pile's base

$$y = 0: \qquad \left.\frac{\partial T}{\partial y}\right|_{y=0} = 0 \, ; \quad \left.\frac{\partial C_{ox}}{\partial y}\right|_{y=0} = 0 \tag{7}$$

Exchange with the surroundings at the outer pile surface should consider heat transfer caused by a combined action due to convection and radiation through impermeable walls:

$$y = H: \qquad -k_{eff} \left.\frac{\partial T}{\partial y}\right|_{y=H} = q''_{conv} + q''_{rad} \, ; \; D_{eff} \left.\frac{\partial C_{ox}}{\partial y}\right|_{y=H} = 0 \tag{8}$$

$$-k_{eff} \left.\frac{\partial T}{\partial n}\right|_{y=H} = q''_{conv} + q''_{rad} \, ; \; D_{eff} \left.\frac{\partial C_{ox}}{\partial n}\right|_{y=H} = 0 \tag{9}$$

$$q''_{conv} = h \cdot \left[T - T_a(t) \right] \tag{10}$$

The heat transfer convective coefficient can be assumed to change with ambient air velocity v (Duffie & Beckman, 1980):

$$h = 5.7 + 3.8 * v \tag{11}$$

External thermal radiation incorporating incoming daily solar radiation and nocturnal heat losses can be calculated in terms of a function that periodically changes with time according to

$$q''_{rad} = a \cdot sen(w \cdot t) \tag{12}$$

The pile dimensions indicate that an idealized two-dimensional domain is appropriate to assess the effects of pile geometry. Three cases were considered for the compost piles investigated: 1) symmetric trapezoidal; 2) asymmetric trapezoidal; 3) fifth polynomial contour.

1.5 Numerical prediction using the Finite Volume Method.

The system of equations that governs this problem (1-12) can be solved numerically using the finite volume method, Patankar (Patankar, 1991). Each one of the governing equations was written in the general form of the transport equation, with unsteady, diffusion and linearized source terms:

$$\frac{\partial \phi}{\partial t} = div\left(\Gamma \cdot grad\phi\right) + Sc + Sp \cdot \phi \tag{13}$$

First-order accuracy in time was used in the numerical scheme to account for the unsteady heat and mass terms:

$$\frac{\partial \phi}{\partial t} = \frac{\phi^{t+\Delta t} - \phi^{t}}{\Delta t} \tag{14}$$

The diffusion coefficient (Γ) and source terms (*Sc, Sp*) for each dependent variable ϕ are given in Table 1.

ϕ	Γ	S_C	S_P
T	k_{eff}	$\frac{T^p}{\Delta t} + Q_c(1-\varepsilon)\rho_c A_c C_{ox} \exp(\frac{-E_C}{RT}) +$ $Q_b(1-\varepsilon)\rho_b \rho_c (\frac{A_1 \exp(\frac{-E_1}{RT})}{1 + A_2 \exp(\frac{-E_2}{RT})})$	$-\frac{1}{\Delta t}$
C_{ox}	D_{eff}	$\frac{C_{ox}{}^{p}}{\Delta t}$	$-(1-\varepsilon)A_c \rho_c \exp\left[\frac{-E_c}{RT}\right] - \frac{1}{\Delta t}$

Table 1. Diffusion coefficient and source terms for the mathematical model.

An original computer program written in Fortran, with a combination of the TDMA and the iterative Gauss-Seidel method (Patankar, 1991), was used to predict temperature and oxygen concentration inside the compost pile.

The pile geometry was discretized using three uniform grids: 100x100, 200x200 and 300x300 nodes in the x and *y* directions, respectively, to verify that the results obtained were not influenced by mesh size. Numerical simulations were carried out for four piles: 1.7, 1.8, 2.5 and 3.0 m height.

Temperature was noticed to increase initially smoothly with time while oxygen decreased slowly with time until sudden changes, caused by the cellulosic heat generation, were noticed for both temperature and oxygen concentration.

Therefore, a strategy based on the use of dynamic time steps was implemented, with lower time steps when the unsteady terms were higher. The different values of the time steps used in the unsteady calculations were in the interval

$$300s \le \Delta t \le 3600s \tag{15}$$

The iterative procedure ended at each time step when the maximum difference between iteration for $\phi = T, C_{ox}$ satisfied, at each control volume, the convergence criteria

$$\phi_{i,j}^{k+1} - \phi_{i,j}^{k} \le 10^{-4} \tag{16}$$

1.6 Comparison between numerical predictions and experimental results.

In order to validate the mathematical model and the numerical simulations a 2.5 m high, 8.5 m long and 7.0 m wide compost pile was built, with a 3D trapezoidal shape, with a 2.5 m wide top surface. Temperature was recorded using type K thermocouples (Ni-Cr) 0.1 m long and 0.0015 m in diameter, built with a type G junction and a silver end, provided with a 5-m long cable with flexible stainless steel coating. The thermocouples were connected to an LA/AI-A8 Cole Parmer data acquisition system coupled with a PC computer provided with the InstaTrend software programmed to collect temperatures every 10 minutes. Every week the values of the computer collected data were averaged and plotted. The temperature measurement points were located in the pile center (z axis) at 2.1, 1.25 and 0.35 m depth. The pile was built with sewage sludge produced by a municipal wastewater treatment plant located in the city of Santiago, Chile (El Trebal) in a landfill belonging to the same plant. The sewage sludge was produced in July 2004 and was used to build the pile in the third week of February 2005.

The experimental and numerical data are compared in Figure 2 (Escudey et al., 2008). Data obtained from numerical calculations were plotted considering daily output at 12:00 a.m. The experimental and predicted data follow the same general trends. Near the surface (0.35 m depth) during the third week the main differences found were not greater than 3°C. The best description of the experimental data was obtained at a depth of 2.1 m.

1.7 General analysis results for the temperature time evolution in a compost pile.

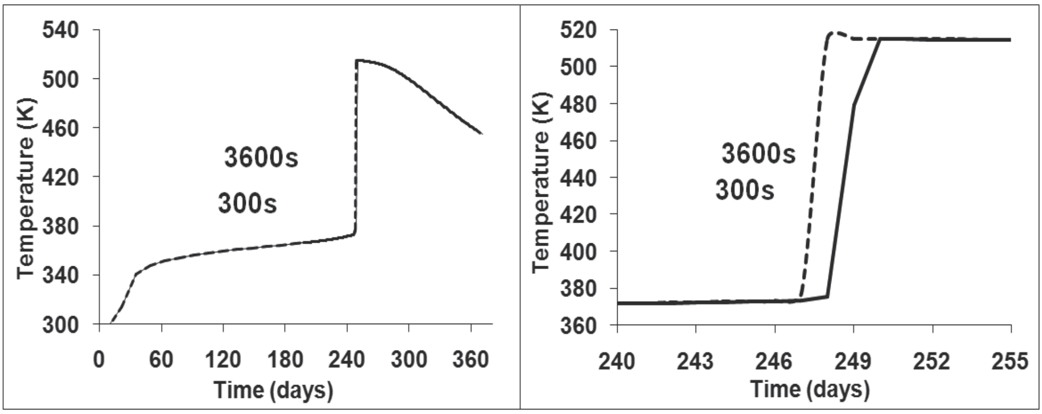
A fundamental process in a compost pile is to achieve a higher enough temperature to cause the death of pathogenic micro-organisms together with the degradation of carbonaceous material. Field practice indicates that an adequate compost process may be achieved when the temperature inside the pile is between 313 K and 353 K. Initially, the temperature inside the pile increases slowly from 293 K up to 353 K due to microorganism growth, as described by the last term on the right-hand side of Equation (1). As the temperature increases beyond 353 K the death of different microorganism colonies is followed by a sudden increase in cellulose oxidation, included as a volumetric heat generation in the second term on the right-hand side of Equation (1). Cellulose oxidation is accounted for in terms of the oxygen concentrations in the heat generation term of Equation (1) and of its counterpart term of oxygen depletion in the last term of the mass diffusion Equation (2). The sudden temperature increase is known as a thermal explosion.

The compost's physical properties change dramatically when the soil has undergone a thermal explosion and hence the compost material cannot be used as a soil fertilizer. Field control of such a sudden temperature increase is a very difficult task and thermal explosions often occur in practice, with loss of the compost material.

A first case of self-heating in a rectangular porous pile 2.5 m high (H) and 5 m long (L) was investigated using three grids, with 100x100, 200x200 and 300x300 nodes, and three time steps: 300 s, 600 s and 3600 s. The temperature-time evolution was calculated at the three positions: f) H/4, L/4; g) H/2, L/2, and h) 3H/4, 3L/4. The results of the time in days needed to cause self-ignition are shown in Table 2 for the three positions. The use of a time step of 600 s and a grid with 300x300 nodes allows to calculate self ignition times of 247, 246 and 250 days at the three vertical positions, respectively, regardless of the time and space discretizations.

Grids	Δt (s)	f) H/4,L/4	g) H/2,L/2	h) 3H/4, 3L/4
100x100	3600	247	245	249
	600	248	248	250
	300	244	241	246
200x200	3600	248	246	251
	600	250	249	253
	300	246	244	249
300x300	3600	248	247	252
	600	247	246	250
	300	247	246	250

Table 2. Days before self-ignition at positions f), g) and h) within the compost pile.



a) b)

Fig. 3. Temperature evolution in time calculated with two time steps, 300x300 mesh, at position H/4, L/4, for a 2.5m high pile, a) full time scale, b) during thermal explosion.

Figure 3 shows the time evolution results of temperature at the f) position: H/4, L/4, calculated with a grid of 300x300 nodes using two time steps: 300 s and 3600 s. A typical heating curve depicting four stages is observed in Figure 3a. A first phase, lasting 35 days, where temperature increases from 293 K to 340 K, is seen. The growth and activities caused by aerobic micro-organisms, included in the mathematical model by a volumetric heat generation, originated the initial pile heating. A second phase, from day 35 to day 246, with a very slow heating process in which temperature increases from 340 K to 370 K, is originated initially by thermophilic micro-organisms whose number decreases progressively as temperature increases, followed by the cellulosic oxidation of the wood chips (used to increase the pile's mechanical strength), incorporated as the first heat generation term in the right-hand side of Eq. (1). Auto-ignition at H/4, L/4 occurs after 247 days, during the third stage, in which temperature increased suddenly in one day from 370 to 515 K. A complex system of solid, liquid and gaseous fuels as a final result of cellulosic oxidation caused a volumetric heat generation that led to the self-ignition process. Temperature decreasing in time characterized the fourth and last stage, in which the fuel reserves at the location are becoming exhausted.

Figure 3b, a zoom view of Figure 3a for the time interval between days 240 and 255, shows that a time step reduction from 3600 s to 300 s allows a more accurate prediction (within 1 day) of the time needed to initiate the self-ignition in a 2.5 m high compost pile.

From the above analysis, a mesh with 300x300 nodes and a dynamic time step of 300 s during the self-ignition and 3600 s in the other stage are needed to solve the problem in an efficient way.

1.8 Pile height effect on temperature and oxygen concentration

In this section a critical pile height of 1.8 m for the thermal explosion to occur has been found. Heating curves at three different positions (H/4, H/2 and 3H/4) located in the mid horizontal section of four rectangular piles (5 m wide) with 1.7, 1.8, 2.5 and 3.0 m heights are shown in Figure 4. Temperature is observed to increase to 369 K in the 1.7 m high pile, at the H/4 and H/2 vertical locations, and to 353 K at the 3H/4 position after 728 days. A sudden temperature increase that took place at around 240 days for the 2.5 and 3.0 m high piles, and at 397 days (in H/4), 374 days (in H/2) ,and 409 days (in 3H/4) can be seen to characterize the initial time for the self-ignition process. Compost piles with heights greater than 1.7 m were found to initiate self-ignition in a time that is inversely proportional to pile height.

Changes in oxygen content with time, calculated inside the 1.7, 1.8, 2.5 and 3.0 m high compost piles, are described in Figure 5 at three vertical positions (H/4, H/2 and 3H/4). As a result of the coupling between heat and mass transfer caused by the cellulose oxidation in both diffusion equations, sudden oxygen depletions are observed at the 1.8, 2.5 and 3.0 m pile height positions, occurring at the time when fast temperature increases were noticed. When pile height is 1.7 m, oxygen concentration inside the pile remains almost constant during the 728 day period used in the analysis. Oxygen reduction to low levels in the domain, coupled with the high temperatures achieved in the same regions, destroys the aerobic thermophilic micro-organisms.

Table 3 summarizes the results obtained after the self-ignition analysis calculated from the time evolution of temperature and the oxygen concentration distributions inside the four rectangular piles of different heights. Temperature and oxygen distribution results show that when the pile volume to heat transfer external area ratio, V/A, is greater than or equal to 1.05 self-ignition occurs inside the pile.

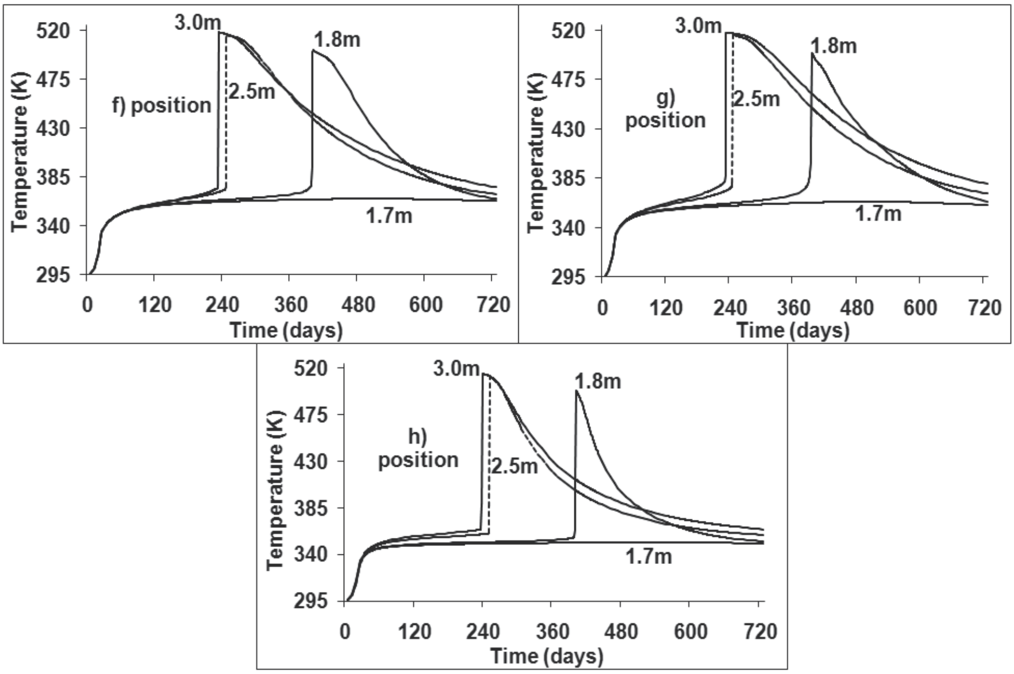


Fig. 4. Time evolution of temperature for four piles with different heights.

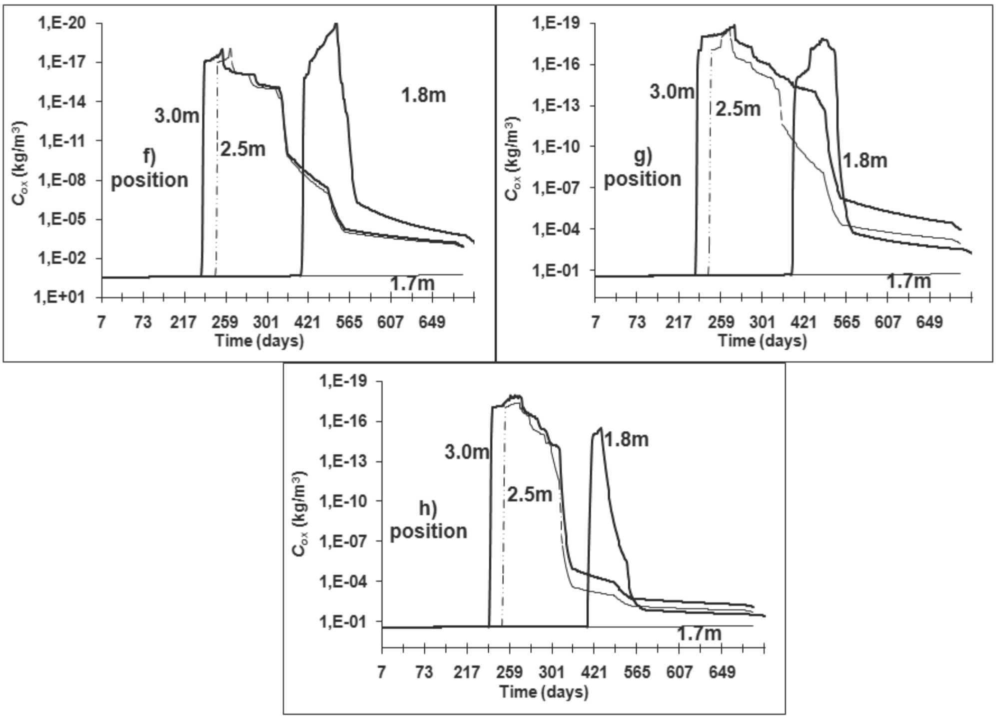


Fig. 5. Time evolution of oxygen concentrations for four piles with different heights.

Pile Height (m)	Area (m^2)	Volume (m^3)	V/A Ratio (m)	Self-Ignition
1.7	8.4	8.5	1.01	no occur
1.8	8.6	9	1.05	occur
2.5	10	12.5	1.25	occur
3.0	11	15	1.36	occur

Table 3. V/A ratio in four piles of different height.

Micro-organism and cellulosic heat generations inside the piles, calculated at a position located at mid section and 3H/4 for four different heights are presented in Figure 6 for the rectangular pile. A rapid initial growth followed by a fast fall in the microorganism volumetric heat generated can be noticed for the four pile heights studied. While cellulosic heat generation inside a 1.7 m pile height is seen to increase to a value that remains almost constant after 450 days, piles taller or equal to 1.8 m show a monotonous increment that is followed by a sudden increment, causing self-ignition. The heat generated by the biological activity of micro-organisms becomes equal to the heat generated by the cellulose oxidation after 102 days for the 1.7 m pile, 105 days for the 1.8 m, 84 days for the 2.5 m, and 77 days for the 3.0 m high piles. In the 1.7 m high pile, after 100 days, cellulosic oxidation keeps the temperatures for all time simulation while the oxygen consumption increases until day 500 and then decreases. Maximum heat generation values for the cellulose oxidations are $2.4x10^5$ (W/m^3), $5.7x10^5$ (W/m^3) and $6.4x10^5$ (W/m^3) for the 1.8 m, 2.5 m and 3.0 m high piles, respectively.

In the water treatment industry the piles are about 1.5 m high and 120 m long. Numerical results verified by experimental observations show that temperature increase in the pile is a function of the volume. Therefore, pile height must be considered a very important factor in thermal explosions. When the initial oxygen content within the pile is at adequate concentrations, microorganism and temperature increases are seen. This occurs until the temperature reaches 353 K, when two phenomena occur: death of the micro-organisms and cellulosic oxidation. The numerical results shown in Figures 6-8 reproduce the biochemical processes described above, resulting in sudden changes in both oxygen and temperature in piles 1.8 m high or higher.

1.9 Compost pile geometric effects.

Heat and mass transfer characteristics are described in Figures 9-11 in terms of temperature and oxygen concentration distribution at four different times in the neighborhood of the self-ignition time. These results were obtained by numerical simulations using the proposed model, equations (1-12). Figure 9 shows the temperature-time evolution and oxygen concentration distributions for a symmetrical trapezoidal pile 3 m high and 8 m at the base, with lateral walls inclined at 45°. A maximum temperature of 440 K was reached after 208 days in the pile's lower-central section, which increases to 519 K on day 216 and is located in the central region at H/3. Temperatures higher than 500 K were measured in the central pile region for 60 days. Oxygen content inside the pile is observed to tend to zero in the lower central section of the pile from 216 to 252 days. Almost the whole pile was seen to be undergoing self-ignition on day 225 except in the regions close to the lateral and top areas.

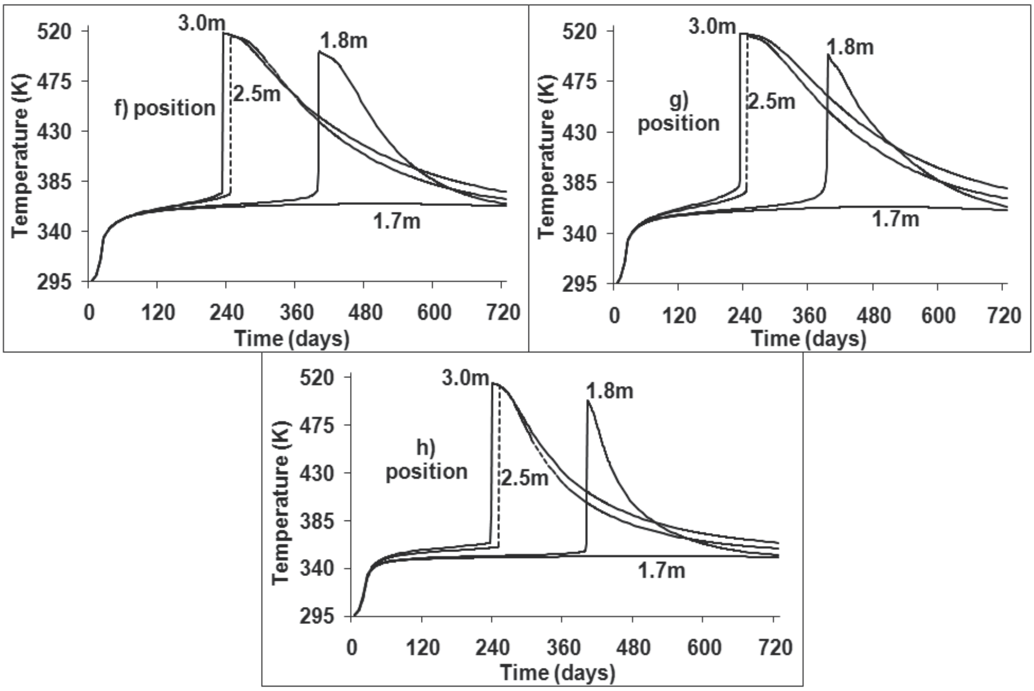


Fig. 6. Time evolution of temperature for four piles with different heights.

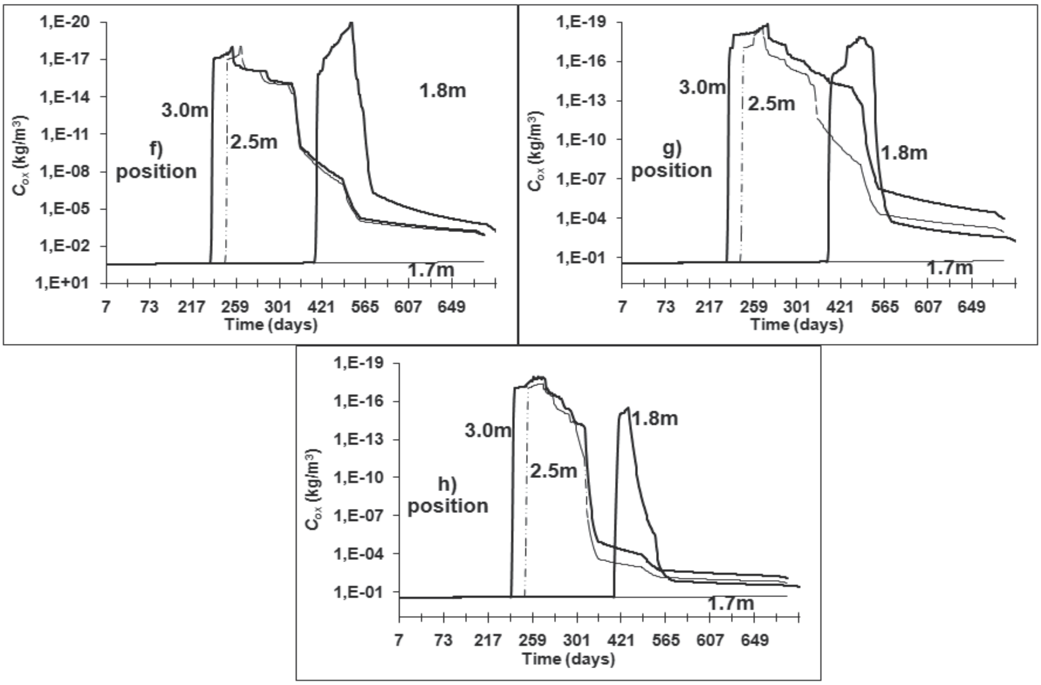


Fig 7. Time evolution of oxygen concentration for four piles with different heights.

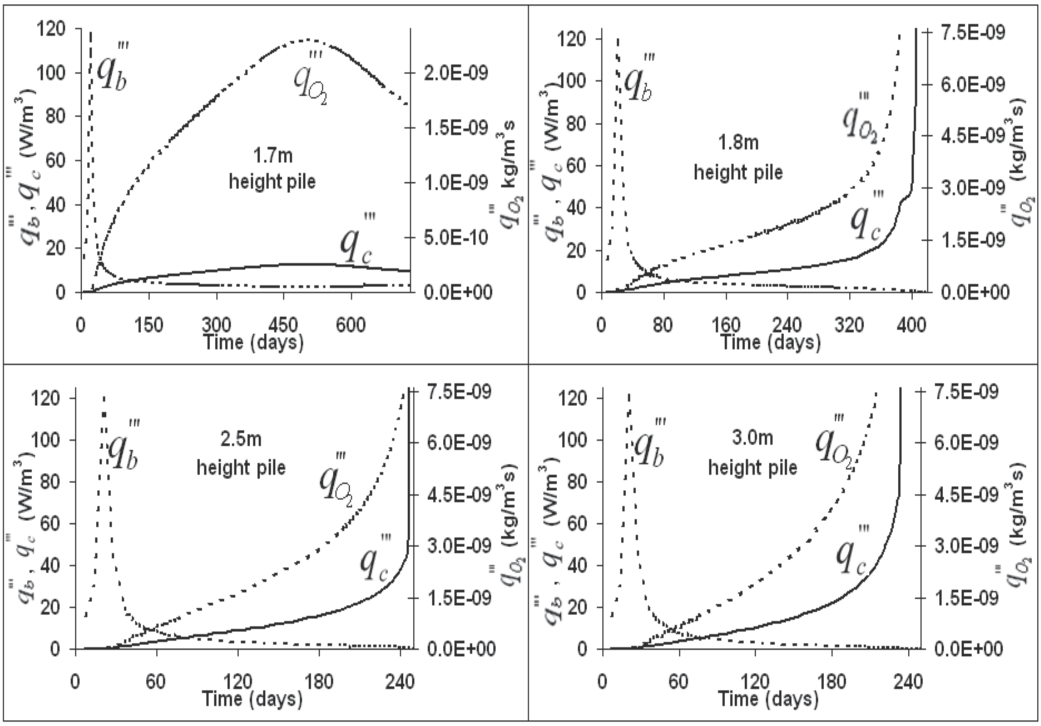


Fig. 8. Time evolution of energy and oxygen consumption for four piles with different heights.

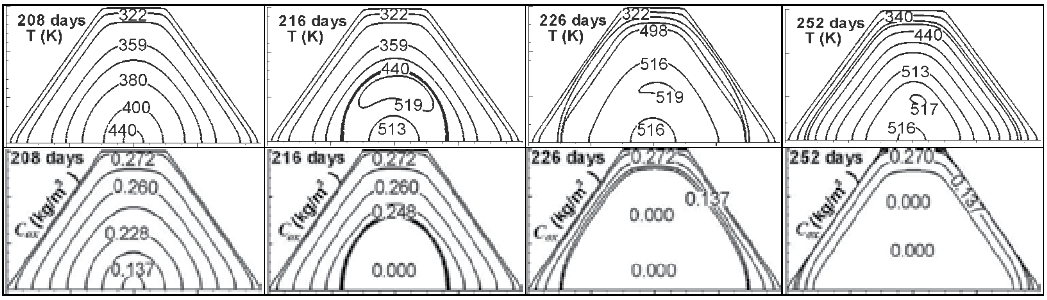


Fig. 9. Distribution of temperature and oxygen concentration within a compost pile, trapezoidal geometry.

Heat and mass transfer inside a non-symmetric trapezoidal sludge pile with an 8x8 m base and lateral walls inclined at angles of θ = 56.3° and β= 33.7° caused by chemical and biological reactions, are described in Figure 10 in terms of temperature and oxygen distribution. Self-ignition started on day 215, when a maximum temperature of 513 K was achieved. A narrow region with high temperature gradients can be observed in the lower central region of the pile on day 217. At this time the self-ignition front is closer to the bigger lateral wall (with β = 33.7°), and therefore smoke production can be expected to begin there. During self ignition the zone with maximum temperature, between 516 and 519 K, reached between days 217 and 253 is closer to the shorter lateral wall.

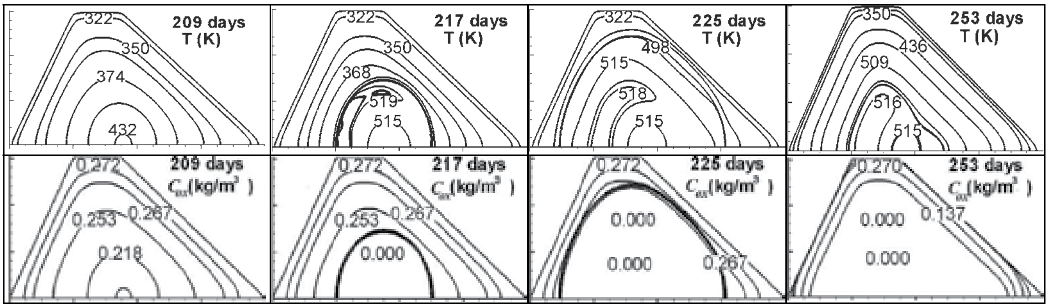


Fig. 10. Distribution of temperature and oxygen concentration within a compost pile, asymmetric geometry.

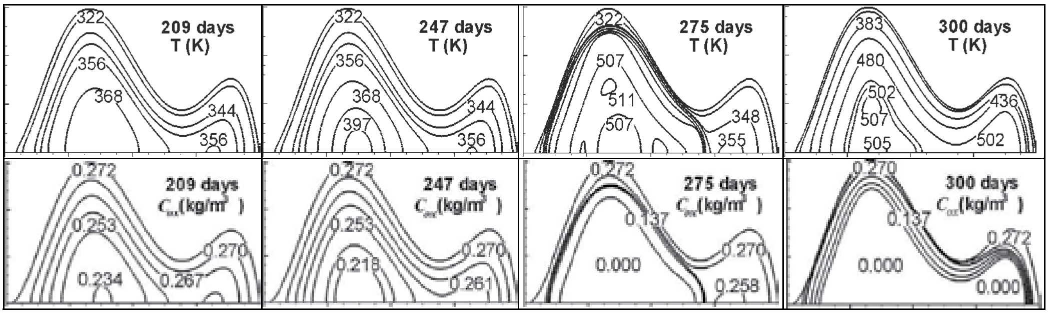


Fig. 11. Distribution of temperature and oxygen concentration within a compost pile, polynomial geometry.

Time evolution of temperature and oxygen concentration distributions at 209, 247, 275 and 300 days for an asymmetrical compost pile with two different height bumps, with maximum heights of 3 m and 8 m at the base, are shown in Figure 11. Self-ignition occurs near the base of the taller region on day 258, with a maximum temperature of 493 K that propagated towards the central zone of the taller region and then migrated towards the pile section with lower height (1.5 m), where a maximum temperature of 502 K can be noticed on day 300. The self-combustion zone can be easily detected as the region in which the oxygen content is zero and on day 300 it can be seen to extend from the pile's base to a region close to the external walls.

1.10 Flow in compost pile as an unsaturated porous medium

The Richards equation (RE) (Richards, 1931) is a standard, frequently used approach for modeling and describing flow in variably saturated porous media. RE is obtained by combining Darcy−Buckingham's law with the mass conservation or continuity equation, under the assumption that the air phase remains at constant (atmospheric) pressure and the water phase is incompressible. Using one dimensional flow in a vertical direction, y, as an example, the following equations depict Darcy's and continuity equation, respectively.

$$q_D = -k_h \frac{\partial \Psi}{\partial y} = -k_h \frac{\partial(\psi + y)}{\partial y} = -k_h \left(\frac{\partial \psi}{\partial y} + 1 \right) \tag{17}$$

$$\frac{\partial\theta}{\partial t} = -\frac{\partial q_D}{\partial y} \pm S \tag{18}$$

where q_D is the flux density (m/s), k_h is the hydraulic conductivity (m), Ψ is the head equivalent of hydraulic potential (m), is the head pressure (m), θ is the volumetric water content (m^3/m^3), y is the vertical coordinate, t is time (s) and S is the source term. Substitution of Equation (17) into (18) gives the mixed formulation of RE:

$$\frac{\partial\theta}{\partial t} = \frac{\partial}{\partial y}\left[k_h\left(\frac{\partial\psi}{\partial y} + 1\right)\right] \pm S \tag{19}$$

Introducing a new term, D(θ) into (19) gives the soil moisture based form of RE. D(θ) is the ratio of the hydraulic conductivity, and the differential water capacity is therefore defined as

$$D_{(\theta)} = \frac{k_h}{\frac{\partial\theta}{\partial\psi}} = k_h\frac{\partial\psi}{\partial\theta} \tag{20}$$

D(θ) is a function of moisture content. This dependence is obtained from field tests.
Combining Equations (19) and (20) gives the θ − based form of RE:

$$\frac{\partial\theta}{\partial t} = \frac{\partial}{\partial y}\left(D_{(\theta)}\frac{\partial\theta}{\partial y}\right) + \frac{\partial k_h}{\partial y} \pm S \tag{21}$$

If the gravitational and the source term effects are neglected, the $\frac{\partial k_h}{\partial y}$ the and S terms in Equation (21) are equal to zero.

$$\frac{\partial\theta}{\partial t} = \nabla D_{(\theta)}\nabla\theta, \tag{22}$$

The volumetric water content is the quotient between water volume and total sample volume, so it has no units and its values are between 0 and 1.

The 1D mass transfer of water in soil solution of Equation (22) for volumetric water content diffusion, testing the effects on the thermal properties caused by moisture in porous media, has been reported by Serrano (Serrano, 2004). This diffusivity coefficient of water in a compost pile is calculated by a nonlinear equation:

$$D_{(\theta)} = \vartheta_1 e^{\lambda(\theta)^{\alpha}} - \vartheta_2, \tag{23}$$

The constants ϑ_1, ϑ_2, λ and α can be obtained by experimental field tests (Serrano, 2004).

Equations (22) and (23) may be used when the specific hydraulic properties of the compost pile are not available.

The effects of the vaporization of water on the internal energy may be calculated by incorporating the third term of the right hand side of Equation (17):

$$-L_v\rho_{va}q(\theta)X_v \tag{24}$$

where L_v is the vaporization enthalpy, $\rho_{v,a}$ is the water vapor density, $q(\theta)$ is the mass water flux, and X_v is the vapor quality.

1.11 Humidity, initial and boundary conditions.

Moisture distributions within the pile are assumed starting from the corresponding first experimental values available:

$$\theta(x, y, 0) = \theta_0 \tag{25}$$

A constant volumetric concentration was imposed at the pile base:

$$\left.\frac{\partial \theta}{\partial y}\right|_{y=0} = 0.6. \tag{26}$$

Heat transfer to the environment when the liquid − vapor phase change takes place was calculated with the equation

$$q''_{w} = h_w L_v(\theta_{w,ml} - \theta_{w,air,ex})\rho_{w,va}, \tag{27}$$

where $\theta_{w,ml}$ is the water content in the fluid adjacent to the surface, $\theta_{w,air,ex}$ is the water content in the outside air, $\rho_{w,va}$ is the water vapor density on the surface, and h_w is the convective mass transfer coefficient. In order to improve the accuracy of the approximation, q''_w were written in the form of a three-point formula (Ozisik, 1994). On the pile's surface h_w and $\theta_{w,air,ex}$ are affected by the distribution coefficient, K, at the interface between the fluid and the solid. Figure 12 shows three concentration points at the interface used for calculating the mass transfer and convective mass transfer at the solid surface using the equilibrium distribution coefficient (Geankoplis, 1993).

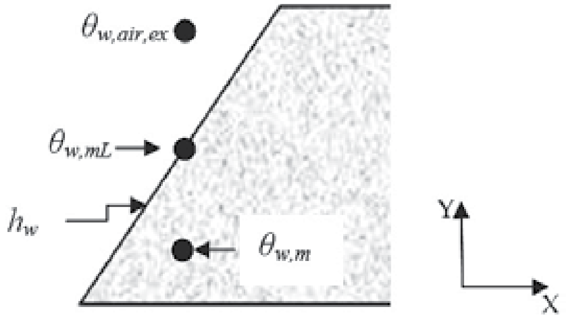


Fig. 12. Source term values for thermodynamic equilibrium

$$K = \frac{\theta_{w,ml}}{\theta_{w,m}}. \tag{28}$$

In Equation (28) $\theta_{w,m}$ is the water content in the solid adjacent to the surface. Substituting Equation (28) in Equation (27) we get:

$$q''_{w} = h_w K L_v\left(\theta_{w,m} - \frac{\theta_{w,air,ex}}{K}\right)\rho_{w,va} \tag{29}$$

The equation for h_w values is obtained as follows (Kaya et al., 2006):

$$h_w = h\left(\frac{D_{w,air}Le^g}{k_{eff}}\right); \qquad g = 1/3; \qquad Le = k/(\rho \cdot C_p) \cdot D_{w,air}\ (Lewis\ number) \tag{30}$$

Water content at the air-compost interface is calculated assuming an ideal gas mixture and molar concentration depending on partial vapor pressure and temperature at the interface

$$\theta_{w,ml} = \frac{p_{va} * 18}{RT\rho_{w,va}}. \tag{31}$$

The vapor pressure is obtained from the relative humidity, $\%H$, as follows:

$$p_{va} = \%H \cdot p^*_{va}, \tag{32}$$

where p^*_{va} is the saturated vapor pressure. Rain effects as boundary condition were incorporated through Equation (20), considering a relative humidity equal to 1 at the surface of the compost pile.

Convective boundary conditions for water content are introduced through equations (33) and (34).

$$D_{w,air} \left.\frac{\partial\theta}{\partial y}\right|_{y=H} = h_w K\left(\theta_{w,m} - \frac{\theta_{w,air,ext}}{K}\right) \tag{33}$$

$$D_{w,air} \left.\frac{\partial\theta}{\partial y}\right|_{y=H} = h_w K\left(\theta_{w,m} - \frac{\theta_{w,air,ext}}{K}\right) \tag{34}$$

1.12 Experimental and numerical results for humidity.

Unsteady water diffusion inside the sewage sludge was investigated by physical experiments and finite volume simulations, based on the mathematical model described by equations (1-5). A compost pile 2.5 m high, 8.5 m long and 7.0 m wide was built, with a 3D trapezoidal shape and a 2.5 m wide top surface.

Figure 13 shows the values measured for rain, wind velocity, and relative humidity at *El Trebal*. In the southern hemisphere February−March correspond to the summer season and April−June correspond to the fall season, where ambient temperature decreases from 293 to 282 K. In this period wind speed drops from about 4.5 to 2 m/s and the relative humidity of the air increases from 54 to 84 percent. The frequency and amount of rain also increase in this period, with maximum values of 40 mm in one continuous rainfall period.

The values measured in the field and those calculated by the FVM (Finite Volume Method) for water content, oxygen concentration, and temperature for each point of measurement are shown in Figure 12. Water content from 0.45 to 0.6 represents optimal conditions for biomass growth. In the field experiment those limiting values were exceeded.

The water content in Figure 14.a is affected by the atmospheric conditions of relative humidity and precipitation, and this is clearly seen at a height of 2 m. Further increases in water content within the compost pile take place when both the relative humidity and precipitation (frequency and quantity) increase. Water content at 0.5 m is less affected by the

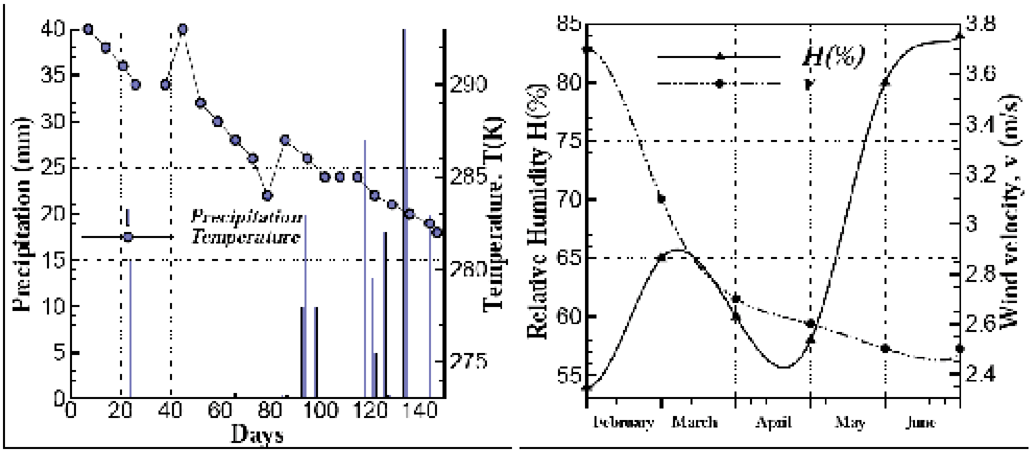


Fig. 13. Precipitation, temperature, relative humidity and wind in *El Trebal*.

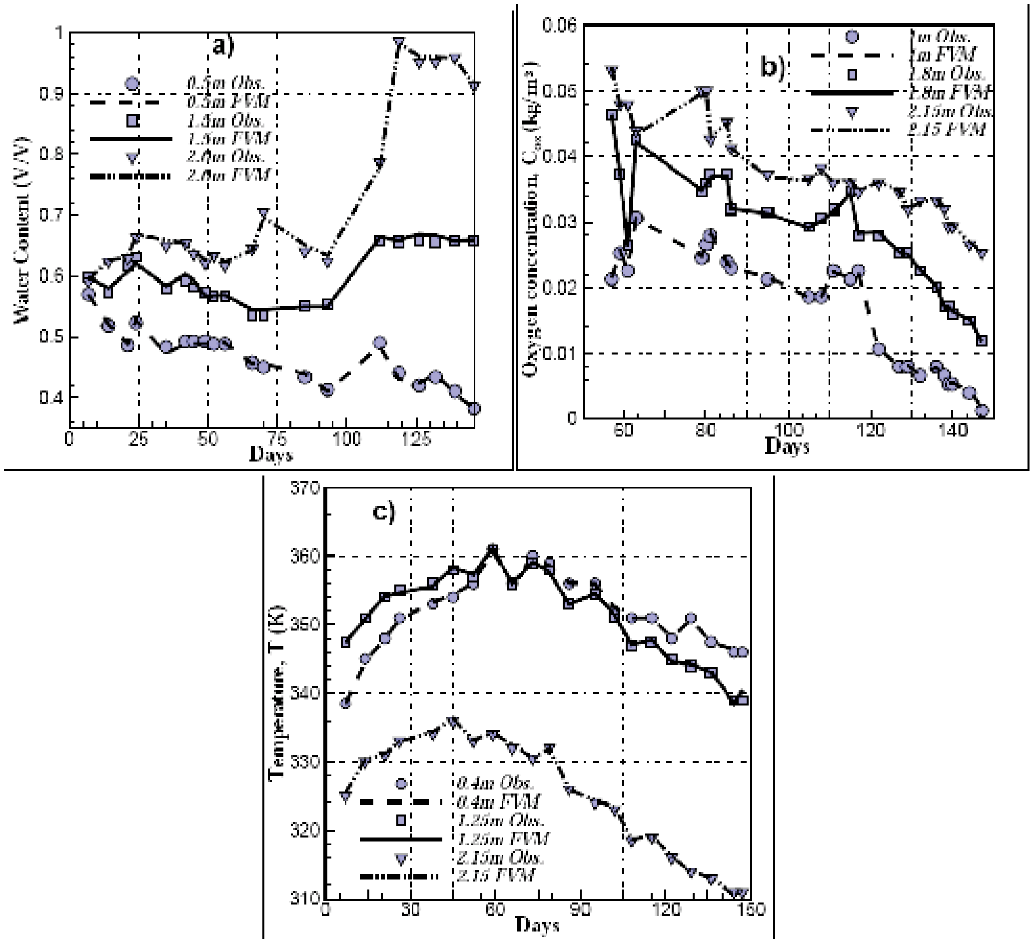


Fig. 14. Water content, oxygen concentration, and temperature observed in the field and calculated with the Finite Volume Methods (FVM) at three heights.

conditions outside the compost pile; at this height the water content is mainly affected by flow into the soil at the pile's base.

In Figure 14.b the oxygen concentration has a tendency to decrease with time. After 115 days the frequency and quantity of rain increase, producing further declines in oxygen concentration because the water displaces the oxygen in the pores. No self ignition conditions were reached in the field during the 21 weeks of the experiment, as shown in Figure 14.c. During the first weeks, temperature in the sewage sludge piles increased up to about 363 K, and it was higher at the first two heights measured within the compost pile. As expected, when temperature in the pile increases, oxygen (C_{ox}) and water content (θ) decrease. Self heating in the compost pile is clearly affected by atmospheric temperature, solar radiation, wind, relative humidity, and precipitation conditions, however further declines in the values are seen after 85 days, caused by the increase in relative humidity and precipitation.

The environment in which the microorganism and chemical reactions occur is altered because of the changes in the moisture and oxygen concentrations, so biological metabolism and chemical reactions decrease, and therefore the temperature within the compost pile also drops. Maximum errors of 0.5K for temperature and 0.0005 m^3/m^3 and kg/m^3 for water content and oxygen concentration between experimental and numerical results were found.

1.13 Conclusions

Numerical simulations indicate that self-combustion does not take place when the piles are less than 1.8 m high, as has been observed in practice. The heat transfer results show that the heating process is initiated by the volumetric heat generation by micro-organisms, and the thermal explosion is caused by cellulose oxidation when the volume to area ratio exceeds 1. The time required to initiate self combustion is inversely proportional to pile height. The internal distribution of temperature and oxygen concentration depends on the geometry of the compost pile. A mathematical model that considers moisture, oxygen and temperature and their corresponding boundary conditions for modeling the compost processes in static compost pile has been proposed.

Numerical simulation with a mesh of 300x300 nodes and dynamic time states between 300 and 3600 s can be used with the Finite Volume Method to predict temperature, oxygen concentration, and humidity within the compost pile.

2. Trace metal leaching in volcanic soils after sewage sludge disposal.

2.1 Introduction

Sewage sludge is the inevitable end product of municipal wastewater treatment processes worldwide. As the wastewater is purified, the impurities removed from the water stream are concentrated. The sludge stream thus contains many chemical and microbiological constituents usually in concentrated forms that may become potential sources of pollutants when the material is released. No matter how many treatment steps it undergoes, at the end, the sludge and/or its derivatives (such as sludge ash) require ultimate disposal, for which the sewage sludge may be land applied, land filled, incinerated, or ocean dumped. There is no entirely satisfactory solution and all of the currently employed disposal options have serious drawbacks. Land application however is by far the most commonly used method around the world. Approximately six million dry tons of sewage sludge are produced annually in the United States (Bastian, 1997). A recent report showed that the annual production of sewage sludge in member countries of the European Union may reach as much as 8 x 10^6 tons (Bonnin, 2001). Significant amounts of sewage sludge produced in the United States and the western European nations have been applied on land. Depending on the regions, 24 to 89% of the sludge produced in the U.S. (Bastian, 1997)has been applied on land. Bonnin (Bonnin, 2001) reported that 65% of the sewage sludge in France was land applied. The situations in other parts of the world are expected to be similar.

As the residue of municipal wastewater treatment, sewage sludge represents the aggregation of organic matter, pathogens, trace elements, toxic organic chemicals, essential plant nutrients, and dissolved minerals originally dispersed in the wastewater that are captured and transformed by the wastewater treatment processes. Properly managed, the potential pollutants are assimilated via the biochemical cycling processes of the receiving soils in the land application. The practice provides soils with organic materials and offers the possibility of recycling plant nutrients, which in turn improve the fertility (Walter & Cuevas, 1999) and physico-chemical properties of agricultural soils (Illera et al., 2006). If not appropriately controlled, the potential pollutants released through land application may degrade the quality of downstream water bodies, be transferred through the food chain to harm the consumers of harvests, and drastically alter the physical and chemical properties of the receiving soils. It is imperative for mass input to provide adequate amounts of substances that are useful for plant development and for pollutant inputs to be controlled to avert detrimental public health and environmental effects. Major countries such as the United States, the European Union (www.europa.eu.int/comm/environment/sludge) have enacted regulations or issued guidelines that limit the disposal options for a variety of reasons. As already mentioned, municipal sewage sludge contains organic matter, essential plant nutrients, and dissolved minerals, and has buffering capacity (Eriksson, 1998; Zhang et al., 2002a, 2002b; Escudey el al., 2004a, 2004b; Pasquini & Alexander, 2004). When land-applied, they may replenish the depleting nutrient reservoirs in these soils under cultivation, allowing the recovery of soil organic matter lost either during a forest fire or in degradation processes due to adverse environmental conditions and unsuitable agricultural practices (Margherita el al., 2006), but they may also involve the input of variable quantities of heavy metals.

In the sewage sludge used, the levels of heavy metals follow the sequence Zn>Mn>Cu>Cr>Pb>Ni>Mo>Cd (from 1780 mg/kg for Zn down to 5 mg/kg for Cd), with land application ass one of the primary options under consideration at this time. In this sense the evaluation of the total metal content in soils or sewage sludge is useful for a global index of contamination, but it does not provide information about pollutant chemical fractions. On the other hand, it has been widely recognized through biochemical and toxicological studies that the environmental impact of heavy metal pollution can be related to soluble and exchangeable fractions that determine bioavailability, mobility, and toxicity in soils (Rauret, 1998; Lock & Janssen, 2001; Guo et al., 2006a). In soils with a mineralogy dominated by crystalline compounds and with lower organic matter content than volcanic soils, it has been found that a negligible movement of trace metals through the soil profile occurred after 17 years of sludge application (Sukkariyah et al., 2005), and Chang (Chang et al., 1984) found that >90% of metals such as Cd, Cr, Cu, Ni, Pb, and Zn added in that way remained in the surface layer (0-0.15 m) after 6 years. Unlike others contaminants, most metals do not undergo microbial or chemical degradation in the soil; therefore, metal concentrations will remain without significant changes for long periods of time (Guo et al., 2006b).

2.2 Impact on Soils from sewage sludge

In Chile, the treatment works are gradually being brought online in recent years. Before that the collected wastewater was discharged directly and sewage sludge did not exist. With the start of wastewater treatment, sewage sludge and ash from the incinerated sewage sludge are accumulating in the metropolitan areas awaiting final disposal. On the other hand, the soils that would be most affected by these amendments are, of course, those that represent about 70% of the country's arable land, soils derived from volcanic ash. The predominant minerals of these soils are allophane and ferrihydrite in the andisols and kaolinite, halloysite and iron oxides in the ultisols. These soils are rich in iron oxides and organic matter, and they have pH-dependent variable surface charge and high PO_4 accumulation. However, the soils have poor fertility; at the original acidic pH range of 4.5 to 5.5 they have low capacity for exchangeable cations (CEC) and a strong selectivity for K and Ca over Mg (Escudey et al., 2004b). Phosphorus is strongly fixed by the minerals, and therefore it is not readily available for plant absorption in these soils. To be productive, they require frequent adjustments of pH, replenishment of exchangeable Mg, and heavy PO_4 applications. When soil pH increases the CEC increases, P fixation decreases, and K selectivity is reduced. On the other hand, when the soil's organic matter increases, K selectivity is also reduced (Escudey et al., 2004b).

In relation to the impact of biosolids, either in their initial state or as ash, studies in pots and columns have been made on soils derived from volcanic materials. In this sense, forest fires are frequent in central-southern Chile; high temperatures may affect heavy metal (Cu, Zn, Ni, Pb, Cd, Mo, Cr, and Mn) chemical fractions naturally present in the soils and those coming from sewage sludge amendment. Changes in exchangeable, sorbed, organic, carbonate, and residual heavy metal fractions, evaluated by sequential extraction, were observed after heating at 400°C in two amended volcanic soils. The most significant heavy metals in these samples were Cu, Zn, Pb, and Ni. A significant increase in the total content of organic matter and metal ions such as Zn and Cu was observed in amended soils with respect to controls. In all samples, sorbed and exchangeable forms represent less than 10% of the total amount, while organic and carbonate fractions represent 24% and 48%, respectively. The thermal treatment of amended soil samples results in a redistribution of the organic fraction, mainly into more insoluble carbonate and residual fractions such as oxides. Finally, the thermal impact is much more important in soils amended with sewage sludge if a heavy metal remediation process is considered, reducing the mobility and solubility of heavy metals supported by sewage sludge, minimizing leaching, and promoting accumulation in surface horizons (Antilen et al., 2006).

Column leaching experiments were conducted to test the ability of Chilean volcanic soils to retain the mineral constituents and metals in sewage sludge and sludge ash incorporated into the soils. Small or negligible amounts of the total content of Pb, Fe, Cr, Mn, Cd, and Zn (0 to <2%), and more significant amounts of mineral constituents such as Na (7 to 9%), Ca (7 to 13%), PO_4 (4 to 10%), and SO_4 (39 to 46%) in the sludge and sludge ash were readily soluble. When they were incorporated on the surface layer of the soils and leached with 12 pore volumes of water over a 3 month period, less than 0.1% of the total amount of heavy metals and PO_4 in the sludge and sludge ash were collected in the drainage water. Cation exchange selectivity, specific anion adsorption and solubility are the processes that cause the reduction of leaching. The volcanic soils were capable of retaining the mineral constituents, P, and metals in applied sewage sludge and sludge ash and gradually released them as nutrients for plant growth.

2.3 Soil description and methods studied

2.3.1 Soils characterization

Soil samples were collected in southern Chile from a depth of 0 to 25 cmin the areas of Collipulli, Ralún, Diguillín, Metrenco, and Nueva Braunau, reflecting the localities from which the soils were extracted. The samples were obtained from well drained and regularly cultivated fields. Collipulli and Metrenco are classified as ultisols and Ralún, Diguillin, and Nueva Braunau as andisols. General information on the climate and geography of the soils is given in Table 4. Also, mineralogical composition can be observed in Table 5. The soil samples were screened in the field to pass a screen with 2 mm openings and stored at the field moisture content in a 4°C cold room until used.

Soil	Location		Soil Order	Classification	Altitude (m)	Rainfall (m yr^{-1})	Annual Mean Temperature (°C)
	Longitude	Latitude					
Collipulli (C)	36°58′S	72°09′W	Ultisol	Fine, mesic, Xeric, Paleumult	120 - 400	1.2 - 1.5	15.8
Metrenco (M)	38°34′S	72°22′W	Ultisol	Fine, mesic, Paleumult	100 - 300	1.2 - 1.5	14.6
Ralun (R)	41°32′S	73°05′W	Andisol	Mesic, Umbric Vitrandept	600 - 1,400	4.0 - 5.0	10.5
Diguillin (D)	36°53′S	72°10′W	Andisol	Medial, thermic, Typic Dystrandept	120 - 180	1.2 - 1.8	15.5
Nueva Braunau (NB)	41°19′S	73°06′W	Andisol	Ashy, mesic Hydric Dystrandept	100 - 150	1.2 - 1.5	11.5

Table 4. Soil classification information

Mineral					
	C	M	R	D	NB
Allophane			+++++	+++++	+++++
α-Cristobalite	+		+		+
Chlorite - Al			++		
Feldspars					+
Ferrihydrite				+	+
Gibbsite					++
Geothite		+			
Halloysite	+	+++++		++	
Kaolinite	+++++				
Montmorillonite			+		
Organo-allophanic				+	++
Plagioclase			++	++	
Quartz		+			
Vermiculite	+			+	

+++++ represents dominant (>50%),
++++ represents abundant (20-50%),
+++ represents common (5 - 20%),
++ represents present (1 - 5%), and
+ represents trace fraction (<1%)

Table 5. Mineralogical composition of soils as represented by the B horizon.

2.3.2 Column experiments

Soils were packed to a depth of 25 cm in 30 cm long and 10 cm diameter acrylic columns, according to their respective field bulk densities. A filter paper disk was placed on the perforated plate at the bottom of each column to prevent the loss of solid materials. The sewage sludge was obtained from a domestic water treatment plant located in Santiago (Chile) and the sewage sludge ash was obtained by heating the sewage sludge at 500°C for two hours. Depending on the treatment, 30 g of air-dried sewage sludge or the ash equivalent of 30 grams of air-dried sewage sludge were placed on the surface 5 cm of the packed columns. The experimental controls received no sludge or ash treatment. The columns, placed vertically, were flooded once a week with one pore volume of distilled water, and drained by gravity from top to bottom, for a period of 12 weeks. Furthermore, 30 g of sludge and the ash equivalent of 30 g of sludge were leached in the same manner. The drained liquid from each weekly leaching cycle was analyzed for pH, electric conductivity, SO_4, PO_4, Na, K, Mg, Ca, Zn, Cu, Fe, Al, Ni, Cd, Pb, Mo, and Mn.

At the end of the leaching experiment, each soil column was cut open lengthwise and the profile was sectioned into five equal length segments for analysis of the soils' pH, electric conductivity, and organic carbon, exchangeable cation and P contents. A chemical fractionation of heavy metals was carried out in sludge and sludge ash using the methodology proposed by Chang (Chang et al., 1984). Sequential extraction with 0.5M KNO_3, distilled water, 0.5M NaOH, 0.05M EDTA, and 0.5M HNO_3 allowed the estimation of the exchangeable, sorbed, organic, carbonate, and residual heavy metal fractions.

2.3.3 Chemical determinations

The bulk density, exchangeable cations, total porosity, and organic carbon content of the soils were determined by methods outlined in Methods of Soil Analysis. Briefly, bulk density (Blake, 1965) was determined by the average air-dried weight of soils in undisturbed soil cores of the 0 to 25 cm soil profile in 5 cm (diameter) x 5 cm (height) brass rings; exchangeable cations were determined as the concentrations of Na, K, Mg, and Ca in ammonium acetate extracts (Peech, 1965); and organic carbon was determined by the Walkley-Black method (Allison, 1965). The pH and electric conductivity of the soils were measured in soil suspensions with a1: 2.5 w/v soil-to-water ratio. The total elemental contents of Na, K, Mg, Ca, Zn, Cu, Fe, Al, Ni, Cd, Pb, Mo, Mn, P and S were determined by digesting the soils with a concentrated HNO_3-HCl-HF mixture in a microwave oven and measuring the concentrations by ICP-OES spectroscopy (Perkin Elmer Optima 2000 equipment). Comparable components of the sewage sludge and sludge ash were determined in the same manner. The concentration of the same elements in leachates was also determined by ICP-OES; SO_4 and PO_4concentrations in the drainage water were measured by ion chromatography (Waters 625LC) in a Waters IC Pak anion HR 4.5x75 mm column. The absorbance of leachates was measured at 465 and 665 nm in a UV-Visible Perkin Elmer Lambda 20 spectrophotometer.

Prior to the sludge and ash treatments, the soils were acidic, with pH varying between 4.5 and 5.9, and low in exchangeable base contents varying from 1.5 to 10.4 cmol kg^{-1} (Table 6). In contrast, the sewage sludge and sludge ash had pH 7.7 and 7.4, respectively, 2 to 3 orders magnitude higher in alkalinity than the soils. The exchangeable base content of the sewage sludge was 80.6 cmol kg^{-1}, 10 to 54 times higher than that of the soils. The Na, K, Mg and Ca in the sludge ash were soluble but not necessarily in the exchangeable forms. Judging from their electric conductivities, the soluble mineral contents of sewage sludge and sludge ash were orders of magnitude higher than those of the soils, even though the incineration of sewage sludge results in less soluble chemical forms, and consequently presents a lower electric conductivity than the sewage sludge. The total elemental Ca, Mg, K, and Na content in the soils follows the same trends as that in the exchangeable forms and the concentrations are in the same order of magnitude. Column pore volume was calculated considering the amount of soil in the column and the total porosity of each soil (Table 6).

Soil	pH	Bulk Density ($g\ cm^{-3}$)	Pore Volume (mL)	Organic Carbon (%)	Electrical Conductivity ($\mu S\ m^{-1}$)	Exchangeable Bases (cmol kg-1)			
						Na	K	Mg	Ca
Collipulli (C)	5.4	1.36	1027	2.3	81	0.1	0.2	1.8	5.9
Metrenco (M)	5.5	1.33	1056	1.8	29	0.2	0.3	1.5	4.0
Ralún (R)	4.5	0.90	988	6.2	436	0.1	0.1	0.4	2.5
Diguillin (D)	5.9	1.12	830	6.5	94	0.2	0.7	1.1	8.4
N.Braunau (NB)	5.5	0.82	834	11.0	20	0.1	0.1	0.2	1.1
Sludge	7.7	0.46	-	17.8	8520	1.5	2.5	10.7	65.9
Sludge Ash	7.4	-	-	<0.1	3890	1.2	1.1	7.4	25.8

Table 6. Properties of soils, sewage sludge and sludge ash.

2.1.4 Releases from sludge and sludge ash

When the sludge and sludge ash were leached, soluble species such as K, Na, Ca and Mg appeared in the leachates. In general, the behavior observed for the K, Na and Mg species indicates a gradual and constant elution, with an important removal in the first pore volume, considering that the curves describe the accumulated amount of exchangeable bases. Comparatively, Fig. 15 shows greater elution from the sludge than from the sludge

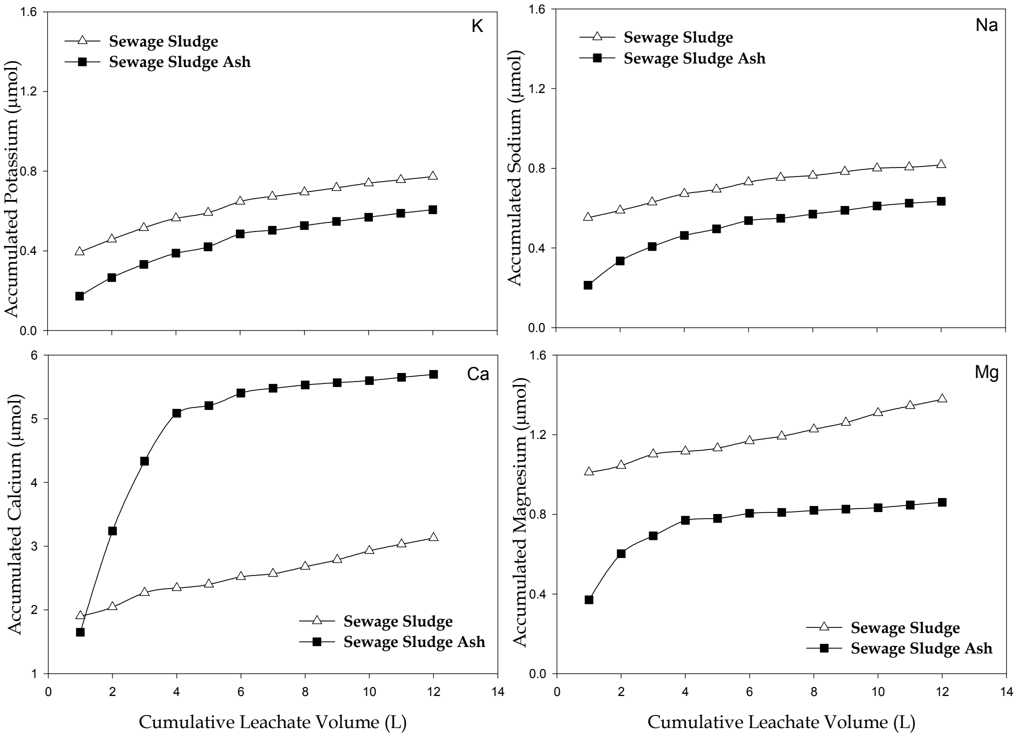


Fig. 15. Accumulated exchangeable bases (K, Na, Ca and Mg) from sewage sludge and sludge ash.

ash, except for Ca. In that relation Ca also presents the greatest elution in the first four pore volumes, exceeding largely the elution from the sludge. This behavior is related to the addition of lime that is made in the water treatment plants with the purpose of stabilizing the pH of the residues. Another species of interest is sulfate, where the soluble SO_4 in sewage sludge was depleted with one pore volume of water used to leach the soils. In contrast, the soluble SO_4 in sewage sludge ash is gradually released with 5 to 8 pore volumes of water, with total amounts released of 342 and 319 mg, respectively.

One main domain is observed in sludge release which is associated to highly soluble forms. On the other hand, two main domains are observed in sewage ash, the first associated with soluble forms which are less important than in sludge, and a second from 2 to 5 pore volumes which can be associated with slow equilibrium between solid and water. In both samples the quantities released were a small fraction of the total amounts.

Only small amounts of K, Na, Ca, Mg and SO_4 were released when the sludge and sludge ash were subject to intense leaching for 12 weeks.

In Chile, the total metal content in the sewage sludge follows the sequence Zn > Cu > Pb > Ni. Fractionation data show that Zn and Cu are mainly associated with highly insoluble fractions, such as carbonates and residual fraction. In control soils the total heavy metal content follows the sequence Zn > Cu > Ni > Pb for Collipulli soil, and Cu > Zn > Ni> Pb for Ralún soil (Antilen et al. 2006). On the other hand, the Zn and Cu release patterns for the sludge and sludge ash were similar (Figure 16), with the accumulated amounts released by the sludge considerably higher than those of the sludge ash.

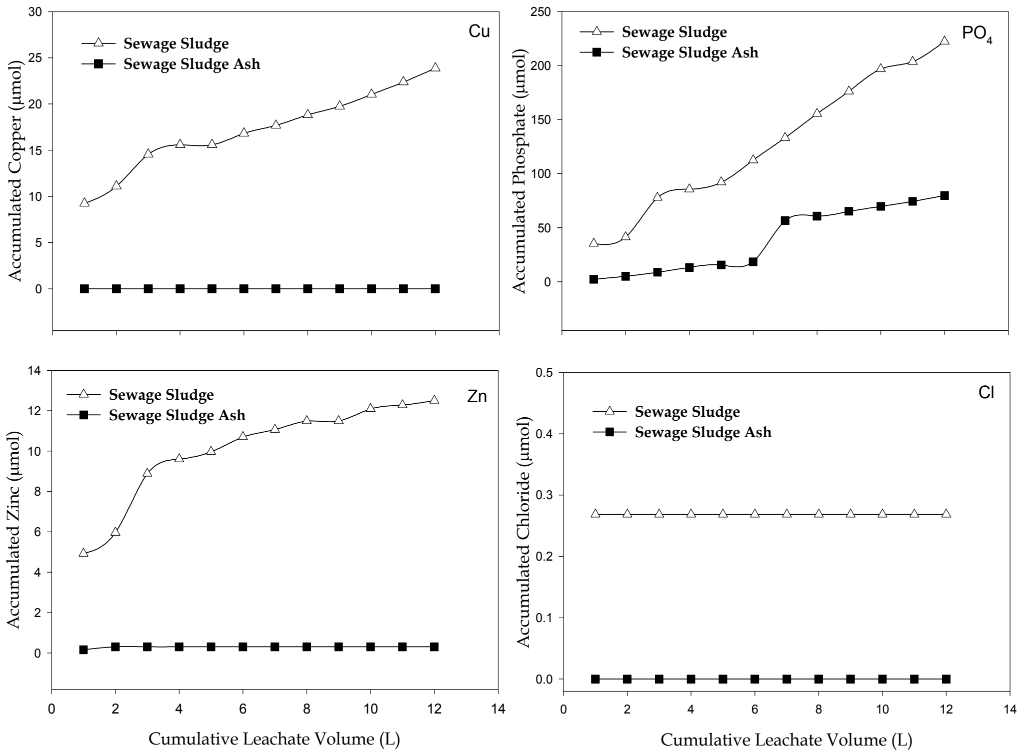


Fig. 16. Accumulated releases of heavy metals (Cu, Zn), phosphorus (PO_4) and chloride (Cl).

In relation to organic and inorganic P forms, both are present in sludge, while in sludge ash only inorganic P forms are present. The P forms in both samples are released slowly and at constant rates over time. In sludge, release is probably controlled by slow equilibrium between solid organic P forms and soil solution, and by the solubility of inorganic P forms. Consequently, at the end of 12 leaching cycles, small amounts of PO_4 were recovered from the drainage liquids of sewage sludge and sludge ash (18 and 6 mg, respectively) compared with their total contents (181 and 170 mg, respectively).

Even though Cu and Zn are the main heavy metals in Chilean sewage sludge, other heavy metals of environmental interest, such as Ni, Cd, Cr, Mo and Mn, were also considered. The total eluted amounts of some of these metals are shown in Table 7, where it is clear that it is minimal compared to the content in the sludge.

	Total leached amount (µmol)						
	Cr	Ni	Mo	Mn	Cd	Cu	Zn
Sewage sludge	1.00	6.07	1.61	9.15	0.00	0.00	0.789
Sewage sludge ash	0.32	0.11	2.47	1.86	0.00	1.61	0.02

Table 7. Accumulated heavy metals leached with12 pore volumes from sewage sludge and sludge ash

2.4.1 Soil attenuation

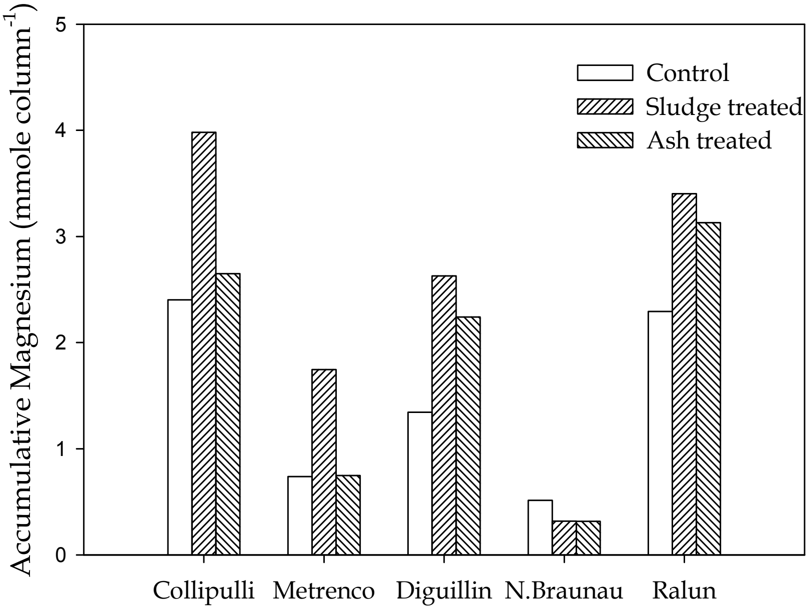
The pH of leachates in the control and treated soils increases after 12 pore volumes; the final pH is about 1.5 to 2.0 units higher than the initial pH. The process is controlled by the soil; thus, after 12 pore volumes the pH of treated soil leachates is only about 0.3 pH units higher than those observed in the control columns. In all the experiments, after 12 pore volumes, the leachate pH is basic, ranging from 7 to 8.

The leaching of organic matter was followed by measuring the absorbance of the leachates after each pore volume at 465 and 665 nm. Only leachates from Ralún soil columns showed absorbance higher than zero, but the amount of organic matter leached was too low to be quantified. No significant loss of organic colloids was observed, because the mass balance shows that the organic carbon remains constant in all columns considering the experimental errors of the Walkley-Black method.

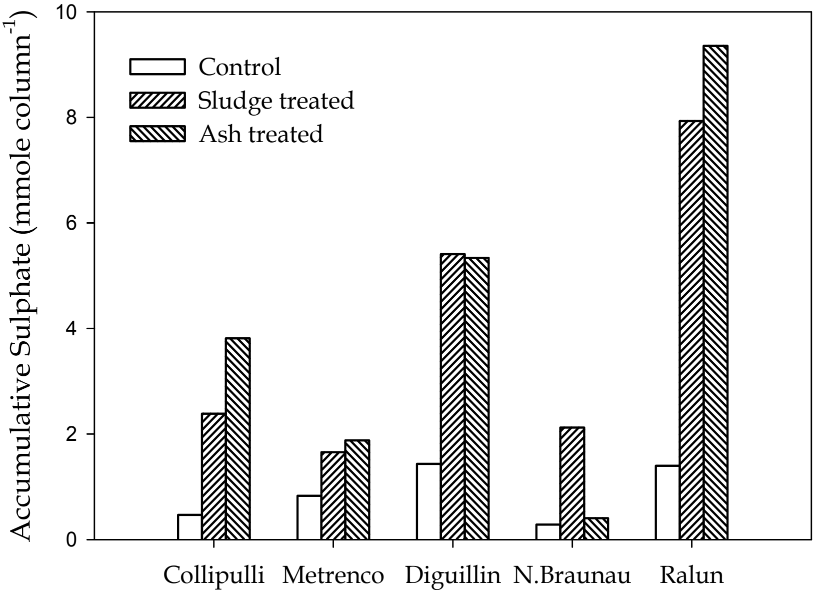
Even without the applications of sludge or sludge ash, cations and anions such as Mg, and SO_4 may be leached from the soils (Figure 17) and the amounts collected in the drainage water were dependent on the conditions of the soils. Sludge and sludge ash amendments consistently enhanced the leaching of minerals. However, the collected amounts were significantly smaller than the total introduced through the addition of sludge or sludge ash, and are practically leached in the first 3 or 4 pore volumes of drainage water.

Soil incorporation further reduced the mobility of the chemical constituents in the sludge and sludge ash (Figure 18). For P, the amounts found in the drainage water (Figure 18) were 2 to 3 orders of magnitude lower than the amounts present in the added sludge and sludge ash.

As a result, nutrients such as available P significantly increased with the application of sewage sludge and sludge ash for both the Ultisol and Andisol (Figure 19). The general trend in all the experiments was that only a small fraction of the total amounts incorporated by the addition of sludge or sludge ash were leached.



(A)



(B)

Fig. 17. Total amount of Mg (A) and SO_4 (B) leached from sewage sludge and sludge ash treated soils.

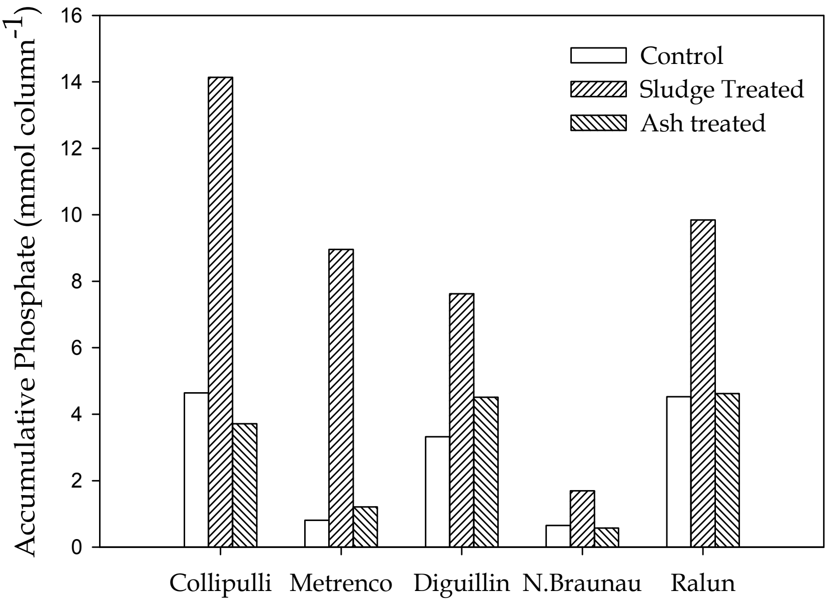


Fig. 18. Total amount of PO_4 leached from sewage sludge and sludge ash treated soils.

As an example, the total input from sludge and ash, the total amount leached from them, and the total amount collected after 12 pore volumes for the Collipulli and Nueva Braunau soils, are presented in Figure 20. The total amount of heavy metals (Cu, Zn, Ni, Cd, Pb, Mo, Mn) leached after 12 pore volumes was <0.1% of the total input from sewage sludge or sewage ash (represented by Zn, Cu and Pb in Figure 20). On the other hand, the leached fractions of SO_4 (22 to 55%), Na (7 to 15%) , K (2 to 30%), Ca (3 to 7%), and Mg (2 to 30%) are more significant.

The leaching of exchangeable bases behaves as predicted by previously reported cation exchange selectivity (Escudey et al. 2002). Phosphate is leached in very low amounts (<0.1%), even though sewage sludge and sludge ash present high P content; this is due to the specific PO_4 adsorption which is a characteristic of Chilean volcanic soils (Escudey et al. 2001).

Fractionation experiments show that 86 to 99% of heavy metal chemical forms in sewage sludge are associated with organic matter complexes, carbonate, and residual low solubility compounds, and that 95 to 99% is associated with carbonate and low solubility forms in sludge ash. All of them have low mobility, and consequently their leaching is mainly associated to the more soluble chemical forms, which are present only in very low concentration in both substrates.

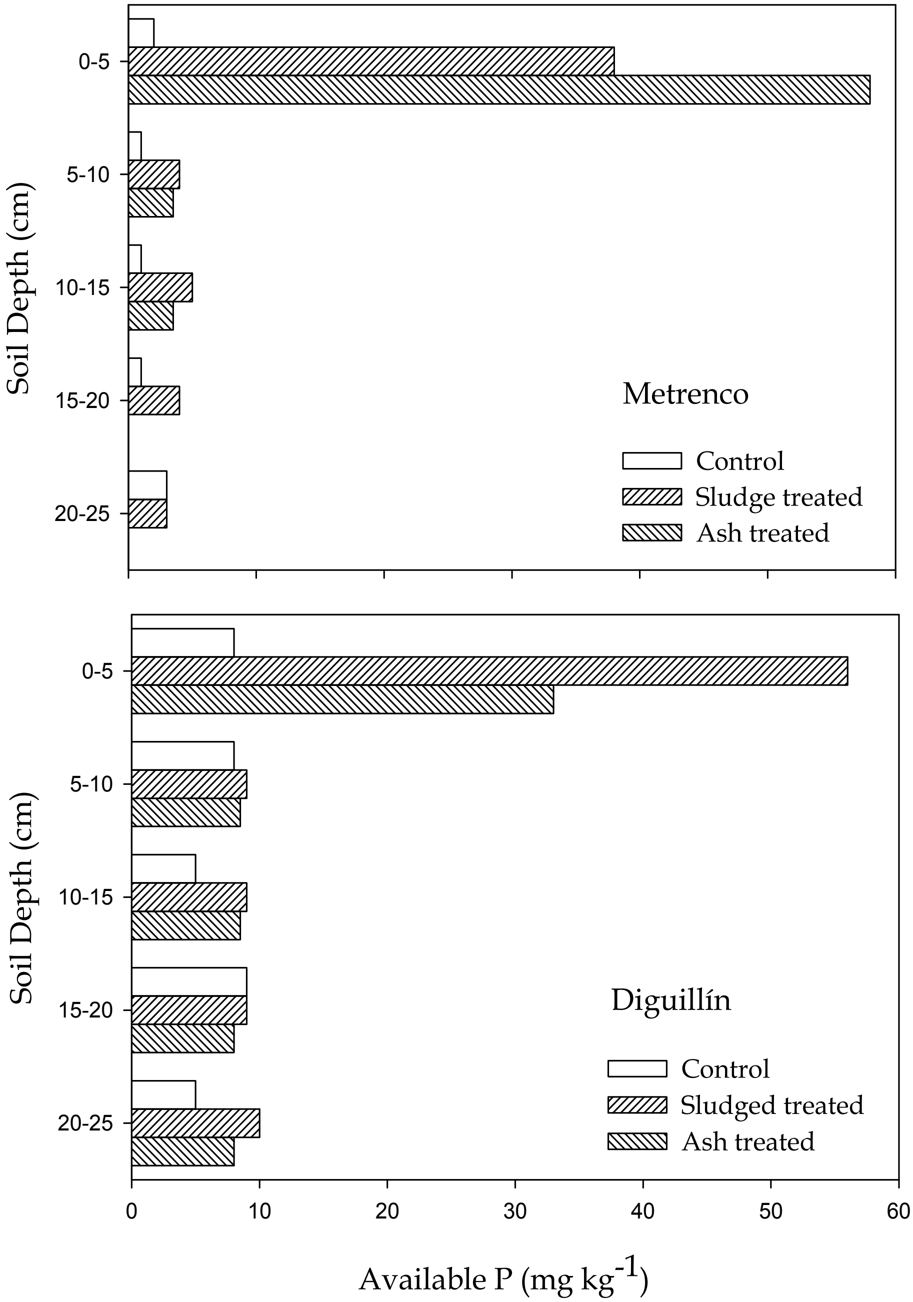


Fig. 19. Available P in the sewage sludge and sludge ash treated ultisol (Metrenco) and andisol (Diguillin).

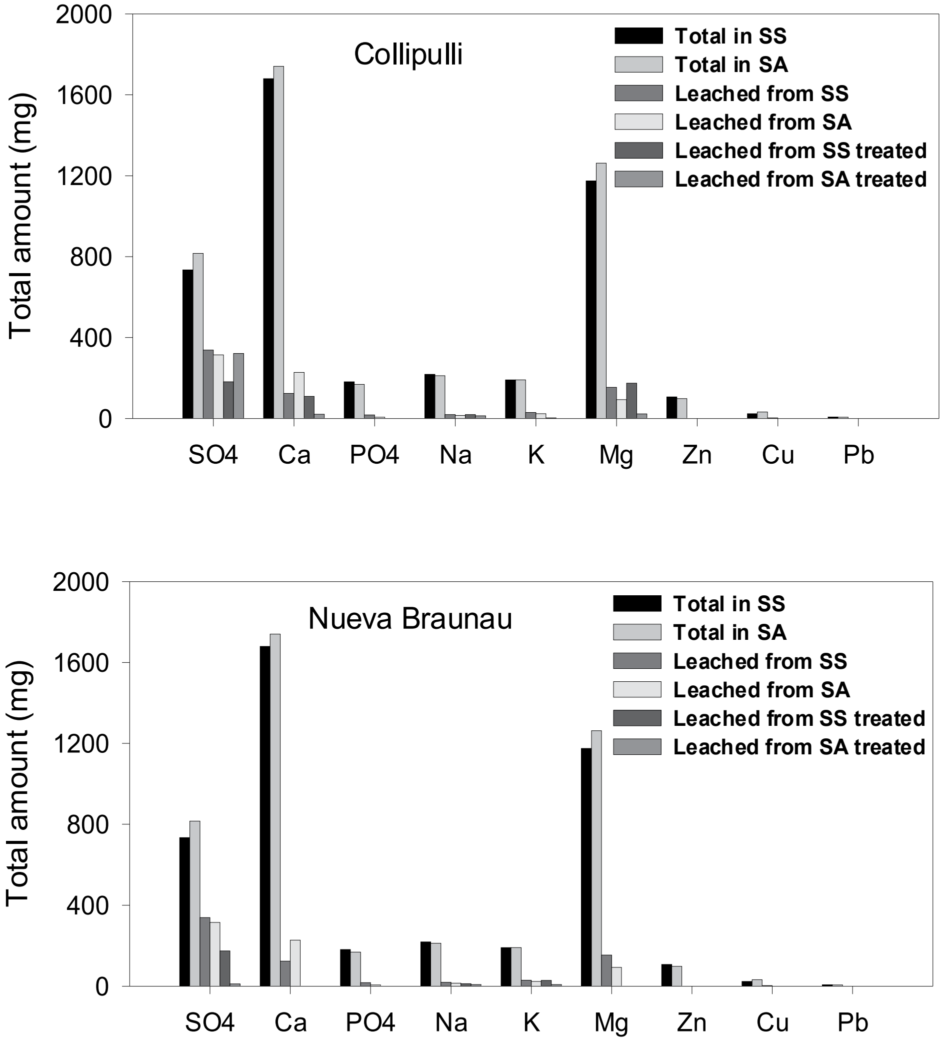


Fig. 20. Total amount of selected cations and anions in sewage sludge and equivalent ash (Total in SS, SA), total amount leached from sewage sludge and sewage ash (Leached from SS, SA), and leached from sewage sludge-treated columns and ash-treated columns (Leached from SS treated, ash treated), for Collipulli and Nueva Braunau soils.

2.5 Conclusions

Results of column leaching experiments showed that volcanic soils in Chile were capable of retaining the inorganic mineral constituents, P, and Zn in sewage sludge and sludge ash when land applied. These constituents are essential inputs to enhance the productivity of volcanic soils that are frequently low in fertility. Cation exchange selectivity, specific anion adsorption and solubility are the processes that cause the reduction of leaching. In this regard, the volcanic soils will attenuate the sewage sludge-borne pollutants and provide soils with nutrients that may be slowly released for crop production.

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22

Waste Water: Treatment Options and its Associated Benefits

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1. Introduction

Wastewater, a semi-liquid waste that is discharged from residential homes, industries, agricultural and commercial properties potentially release significant amounts of toxic and pathogenic contaminants into local treatment plants for processing. These contaminants include not only soaps, shampoos and conditioners used in showers, food scraps and oils from kitchen sinks and garbage disposals, human waste from toilets, detergents and fabric softeners from washing machines and dishwashers as well as all the harsh detergents that clean the house, but also heavy metals, pharmaceuticals, volatile organic compounds (VOCs), volatile toxic organic compounds (VTOCs), pathogenic microorganisms, phosphorus, nitrogen, substances that are carcinogenic, tetragenic and mutagenic that are resistant to typical wastewater treatment processes that come from industries.

Potable water becomes wastewater after it gets contaminated with natural or synthetic microbiological compounds that arise out of human activities, commercial and industrial sources. They may be accompanied with surface water, ground water and storm water. Wastewater is also sewage, storm-water and water that have been used for various purposes around the community. Unless properly treated, wastewater can be harmful to public health and the environment.

2. Sources of wastes

Most communities generate wastewater from both residential and non-residential sources.

Residential Wastewater or Household Wastewater

Residential wastewater is a combination of excreta, flush water and all types of wastewater generated from every room in a house. It is more commonly known as sewage and is much diluted. There are two types of domestic sewage: black-water or wastewater from toilets, and gray water, which is wastewater from all sources except toilets. Black-water and gray-water have different characteristics, but both contain pollutants and disease-causing agents. In the U.S, sewage varies regionally and from home to home. These are based on factors such as the number and type of water-using fixtures and appliances used at homes and even their habits, such as the types of food that are eaten.

Non-Residential Wastewater or Industrial Wastewater

This is mainly made up of wastes coming from commercial activities (e.g., shops, restaurants, hospitals etc.), Industry (e.g., Chemical Industries, Pharmaceutical companies, Textile manufacturing companies etc.), Agriculture (e.g., slurry), construction and demolition projects, mining and quarrying activities and from the generation of energy. These could be places such as industrial complexes, factories, offices, restaurants, farms and hospitals. Because of the different non-residential wastewater characteristics, communities need to assess each source individually to ensure that adequate treatment is provided. For example, laundries differ from many other industrial sources because they produce high volumes of wastewater containing lint fibers. Restaurants typically generate a lot of oil and grease. In addition, many industries produce wastewater high in chemical and biological pollutants that, can overburden onsite and community wastewater treatment systems.

Storm-water is a nonresidential source and carries trash and other pollutants from streets, as well as pesticides and fertilizers from yards and fields. Communities may require these types of nonresidential sources to provide preliminary treatment to protect community systems and public health (Runion, 2010).

Environmental hazards of waste water

Wastewater can attract rodents and insects which cause gastrointestinal parasites, yellow fever, worms, the plague and other unhealthy conditions for humans. Exposure to hazardous wastes, particularly when they are burned, can cause various other diseases including cancers. Wastes can contaminate surface water, groundwater, soil, and air which causes more problems for humans, other species, and ecosystems. Waste treatment and disposal produces significant green house gas (GHG) emissions, notably methane, which are contributing significantly to global climate change.

Disease- causing pathogens are constantly being released into waterways from waste water. However, these chemical substances are only the tip of the iceberg. The pathogens from diseases such as AIDS, cholera, plague, hepatitis, typhoid, polio and so on are also released from homes, medical clinics, laboratories and hospitals. Studies have shown that every gram of fecal material from an infectious hepatitis patient can contain up to 100,000 infectious doses. Other pathogens include *cryptosporidium, giardia, neospora, e.coli, stretococcus, legionella, salmonella, shigella, vibrio*, adenoviruses, Norwalk, rotavirus, amoeba, whipworm, tapeworms, flukes, pinworms, roundworms, *klebsiella, clostridium, pseudomonas* and *mycobacterium tuberculosis*. These microbes are not looked for nor tested in a routine analysis of treated wastewater before their release into the environment.

Many viruses can survive in wastewater up to 41 days at 20°C. Once released into the environment, they can survive up to six or more days in a river and up to 100 days in soil. The protozoa parasite can survive up to 20 days in soil while bacteria can survive up to 120 days. Most worms like the ascaris, tapeworms and trichuris can survive up to 12 months in soil. Their survival in soil depends on moisture, pH, temperature, type of soil and the presence of organic matter (Anon, 1980). It is estimated that every year 1.8 million people die worldwide due to suffering from waterborne diseases. A large part of these deaths can be indirectly attributed to improper sanitation. Wastewater treatment is an important initiative which has to be taken more seriously for the preservation of society both at present and in the future.

Also, Mills discharge millions of gallons of effluent each year, full of chemicals such as formaldehyde (HCHO), chlorine, heavy metals (such as lead and mercury) and others, which are significant causes of environmental degradation and human illnesses. The mill effluent is also often of a high temperature and pH, both of which are extremely damaging. All of the mills O Ecotextiles (A producer of high quality organic fabrics in Seattle, Washington) uses, have wastewater treatment in place. Every 25 meters of an O Ecotextiles sofa fabric prevents 2,300 liters of chemically infused effluent(about the size of a California hot tub and containing from 1 to 10 kg of toxic chemicals), from entering the environment (Based on VPI study for Dept. of Environmental Quality for the state of Virginia.) (Anon, 2005).

Some advantages of waste water and its treatment

Oboh (2005) studied the utilization of fermented waste water from cassava mash as source of industrial amylase and reported that the amylases from fermented cassava waste water are active at wide temperature and pH ranges. This quality could be explored in the industrial sector (most especially food industry) as a source of industrial amylase.

Wastewater treatment is a process whereby the contaminants are removed from wastewater as well as household sewage, to produce waste stream or solid waste suitable for discharge or re-use (Naik, 2010). Treated wastewater is now being considered as a new source of water that can be used for different purposes such as agricultural (70% of Israel's irrigated agriculture is based on highly purified wastewater) and aquaculture production, industrial uses (cooling towers), recreational purposes and artificial recharge. Using wastewater for agricultural production will help in alleviating food shortages and reduce the gap between supply and demand. Treated wastewater can be re-used as drinking water, in artificial recharge of aquifers, in agriculture and in the rehabilitation of natural ecosystems (Florida's Everglades).

Re-use of wastewater, in concert with other water conservation strategies, can help lessen anthropogenic stresses arising from over-extraction and pollution of receiving waters. However, there are concomitant environmental risks with wastewater re-use, such as transport of harmful contaminants in soils, pollution of groundwater and surface water, degradation of soil quality e.g. salinization, impacts on plant growth, the transmission of disease via the consumption of wastewater-irrigated vegetables, and even increased greenhouse gas emissions. The challenge facing wastewater re-use is to minimize such risks so as to maximize the net environmental gain.

There are more than 150 known pathogens detected in untreated wastewater. Every year new ones are being discovered. Of the 72 enteroviruses, many will trigger illnesses that are not gastrointestinal, such as, polio, meningitis, diabetes, muscle diseases and endocarditis (inflammation of the heart muscle that can lead to heart attacks). They can and do produce infectious illnesses in humans that multiply and are re-excreted through fecal material (Mara and Horan, 2003), hence, the need for waste water treatment in order to avoid the occurrence of such pathogens in the environment.

Benefits of treatment on man and the environment

Endocrine disruptors, also known as xenoestrogens, are chemical compounds and by-products used in the plastic, pesticide and chemical industries and found in their waste water that have hormonal effects on the body. There are more than 100,000 registered endocrine disruptors. They are far more potent in mimicking estrogen activity than the body’s natural hormones and far more toxic. The synergistic effects of these chemicals in the body may be up to 1000 times greater. Endocrine disruptors create a large range of reproductive problems. They include infertility, menstrual problems, difficulty holding a pregnancy to term and early puberty. Other health issues include impaired immune function, behavioral problems, brain malfunctions and cancer (Anon, 1995).

Waste water quality deteriorates rapidly and causes microorganisms, protozoa, fecal *coliform*, and fecal *streptococci* in the surrounding area. It is affected directly by the quality of groundwater in the area mainly with surfactants such as Anionic Detergents (linear alkyl sulphonate, LAS) and nitrite in the surrounding area around the infiltration basins with cycle diameter of 800 m. This is very harmful to the human body and cause gastroenteritis troubles.

Wastewater Treatment

Wastewater treatment is the removal of solids, bacteria, algae, plants, inorganic and organic compounds from used water with subsequent conversion into environmentally acceptable water or even drinking water. This treatment usually requires science, engineering, business and art (Anon., 2010) and includes mechanical, physical, chemical and biological methods. Most often, huge amount of waste water is generated from various sources; domestic, municipal and industrial activities. The waste water may be characterized with pH, suspended solids (SS) dissolved solid (DS), turbidity, colour, biological oxygen demand (BOD) and chemical oxygen demand (COD) among others. Each source of the water has its own pollution problem and must be treated to return water to its natural state or re-used in various activities. Waste water treatment can be grouped in three major ways: physical, chemical and biological treatment. Physical treatment involves processes such as sedimentation, aeration, filtration and floatation while chemical treatment uses oxidizing agents such as chlorine, ozone, including neutralization method. Sometimes coagulants like alum, iron (III) sulphate, among others could be utilized. Carbon could be used both in chemical and physical methods as adsorbent. In the biological water treatment, microorganisms such as bacteria are biochemically employed to decompose wastewater to produce water stable product. There are aerobic and anaerobic decomposition. Under aerobic decomposition, contaminants in water are converted to carbon dioxide while anaerobic decomposition produces methane as a fuel in biogas and carbon dioxide as major products. The effluent from anaerobic process can be used as manure in agricultural production. (Anon, 2010).

The Importance/need for Waste water Treatment

Population explosion, disorderly rapid movement to the urban areas, technological and industrial expansion, energy utilization and waste generation from domestic, municipal and industrial sources have rendered many waters unwholesome and hazardous to man and other living things. There are no stringent laws guiding environmental pollution in most developing countries in the world. Consequently, industries discharge untreated or inadequately treated water into water ways (Amuda and Ibrahim, 2006). Most of these waters pose great dangers to aquatic life and must be cleaned or treated.

Reported Researches on Wastewater Treatment Methods

Treatment processes for heavy metals removal from wastewater include precipitation, membrane filtration, ion exchange, adsorption and co- precipitation/adsorption. Studies have shown that adsorption method is highly effective and activated carbon has been in use but very expensive (Nasim *et al.*, 2004). Amuda and Ibrahim (2006), reported on the comparison of adsorption efficiency of coconut shell- based activated carbon and commercial activated carbon calgon, carbon (F-300) in the treatment of industrial wastewater from a beverage industry for the removal of organic matter (COD). The results of their findings indicated that coconut shell- based granular activated carbon was found to effectively adsorb the organic than the commercial activated carbon. Besides, previous researches on the removal of COD from industrial wastewater using coagulation/flocculation, membrane filtration and oxidation processes etc., reveal that the technology of these methods are generally expensive, complicated, time consuming and required skilled personnel (Galambos *et al*., 2004; Peres *et al.,* 2004). However, low cost and non-conventional adsorbents such as nut shells, wood, bone, peat, processed into activated carbons and biomass such as *Aspergillum tereus, Peusodomonas sp*., *Rhizopus arrhizus* are better (Okieimen *et al*., 1985; Tam and Antal, 1999; Bansode *et al*.,2004; Nomanbhay and Palanisamy, 2005; Preetha and Viruthagiri, 2005). Again, ultraviolet (UV) lamp has also been used to treat textile wastewater. Stanislaw and Monica (1999), reported the application two different UV radiations; 150W, λ=254-578nm and 15W, λ=254nm to the synthetic textile wastewater for 1-3h. There was significant inhibitory action on the microbes (47 to 30% reductions) for the optimum radiation time. Moreover, ozone (O_3) is a powerful oxidant for both water and wastewater. The conventional fine bubble contactor is the most widely used Ozone generator because of high Ozone transfer efficiency (90%). Result of findings from Mehmet and Hassan (2002), showed that ozonation using 300mg/dm^3 gave rise to biodegradability index of textile wastewater by 1.6times. Few researchers namely; Namboodri et al., (1994); Rajeswari (2000) among others revealed that ozone decolourizes all dyes except non-soluble dispersed and vat dyes that react slowly and take longer time. Hence, ozone was combined with other agents for its complete oxidation of organic compounds in the wastewater to carbon dioxide and water. This combination has led to advanced oxidation processes using ozone and others, e.g.---O_3/UV, O_3/H_2O_2, O_3/TiO_2, etc. Xuejun *et al., (*2005), used a combination of electrochemical oxidation combined with membrane filtration to treat wastewater from a textile industry. Results obtained showed that electrochemical oxidation has a high removal of 89.9% efficiency of COD of wastewater whereas the membrane filter can almost completely remove total suspended solids (about 100%) and turbidity (98.3%). After electrochemical and membrane filtration steps were employed, COD levels were reduced to 18.2mg/L from 178.5mg/L. Turbidity was reduced from 18.5 NTU to 0.32 NTU. Oparaku *et al*., (2011) reported on disinfection of wastewater from fish pond for re-utilization using ultraviolet radiations. The UV treated wastewater had lowest coliform count of 1.8X10^3 Cfu/ml in comparison with solar and electric powered pump water counts obtained as 2.2X10^3 and 6.8X10^3 Cfu/ml, respectively. This treated water also had dissolved oxygen that amounted to 7.2mg/L, averagely favourable for aquatic life. Moreover, anaerobic digestion has been utilized to convert municipal wastewater (MSW) into methane and carbon dioxide with the effluent used as biofertilizer. Chynoweth *et al*., (1991), presented the results of biochemical potential of several fractions of MSW in order to compare the extent and rates of their conversions to methane. It was discovered that the methane yield was as high as 0.20m^3/kg of volatile solids added. A report from Okafor (1998) showed that cassava wastewater generated from "garri" production (a staple food consumed in the Eastern part of Nigeria), was inoculated with microorganisms to produce microbial biomass. This biomass was then mixed with ground cassava peels to formulate feed for pigs. Droppings from the pigs were later converted to biogas through anaerobic digestion. Again, implementation of anaerobic process on wastewater from tapioca starch industries has been reported. The research results showed that the value of organic substance in the influent were in the average of 10, 062 and 5,649 ppm in terms of COD and BOD, respectively. Maximum organic loading rate applied was 7.8kg COD/m^3 day. The efficiency of degradation reached an average of 76% and 95.8% for COD and BOD, respectively. Methane content in the biogas was in the range of 53.5 to 71% while average biogas production was 1.2m^3/m^3 of wastewater.

3. Anaerobic digestion as a waste water treatment option

Anaerobic digestion (AD) is biological process similar in many ways to composting. It is a natural treatment process and as in composting bacteria in the absence of air, breakdown organic matter and reduce its bulk or mass (polymers) into simpler compounds mainly methane (CH_4) and carbon IV oxide (CO_2) and traces of other gases like O_2, H_2S, NH_3, N_2, CO and water vapour etc (Wolfgang and Axel, 2005). The effluent of this process is a residue rich in essential inorganic elements like nitrogen and phosphorus needed for healthy plant growth known as biofertilizer which when applied to the soil enriches it with no detrimental effects on the environment (Bhat et al., 2001).

Anaerobic waste digestion Takes place in a closed reactor. Bacteria act upon the organic waste and release a lot of methane and carbon dioxide. The microbial community has only obligate anaerobic and facultative bacteria. As in aerobic chemohetrotrophic metabolism, initially the macromolecules are hydrolyzed. These products are then converted to volatile fatty acids (mainly acetic acid), and alcohols. The organisms responsible for these anaerobic waste reactions are popularly called acid formers or acidogens. They obtain energy through oxidation of organic compounds, but do not use oxygen as electron acceptor. Instead, another fragment of the substrate is reduced to anaerobic acids and alcohols through anaerobic processes. These are then metabolized by a second group of obligate anaerobic biomass (the methane formers or methanogens), and are converted to methane gas. It is estimated that 60 to 70% of methane production in an anaerobic waste reaction is through conversion of acetic acid and the rest through carbon dioxide reduction by hydrogen. The activities of the methane and acid- producing groups of bacteria must be balanced as the former is sensitive to pH changes and works best in pH range 6.8 to 7.5. (Runion, 2010).

$$(C_6H_{10}O_5)\,n + nH_2O \rightarrow n\,(C_6H_{12}O_6) \text{ – Hydrolysis}$$

$$n\,(C_6H_{12}O_6 \rightarrow nCH_3COOH - \text{Acetogenesis / Acidogenesis}$$

$$3nCH_3COOH \rightarrow n\,CH_4 + CO_2 - \text{Methanogenesis}$$

The objectives for planning an anaerobic digestion process include

1. To provide waste regulations compliant, sustainable and cost effective method of disposal of organic wastes
2. To treat, clean source separated organic wastes from households, restaurants, industries and other enterprises in an environmentally friendly manner.
3. To provide a sustainable and cost effective method of disposal of ABPR waste materials
4. To reduce carbon emissions and carbon levies payable associated with a business.
5. To establish a sustainable circulation of plant nutrients and organic material between the community and agricultural sector in such a way that the use of the residual is optimized.
6. To provide opportunities for the use of artificial fertilizer (i.e. promote organic farming).
7. To extract and use high grade bio-energy from waste and normal farm crops, with no net contribution to the atmosphere.
8. To promote and develop high efficiency energy processes and remove odours generated from present systems.
9. To reduce risk of water pollution from current practices and generally reduce emissions of enteric organisms and water courses.

Environmental technologists have in the last decade been concerned with straight forward technological, economic challenges such as drinking water production, waste water treatment, refuse handling and treatment , soil and sediment clean-up and waste gas purification. Only recently, they have started to look at their activities from the point of view of sustainability and they have had to admit that in many cases, they were far from holistic (Verstraete and Top, 1992).

Typical examples of non-sustainable approaches are current practices in anaerobic waste water treatment and refuse land filling (Allison-Onyechere et al., 2007). Lettinga et al., 1980 and Verstraete et al., 1996 reported the development of anaerobic sludge blanket or UASB reactor for the treatment of waste water. In this process, the waste water is pumped upwards through a reactor under strictly anaerobic conditions at a rate between 0.5 and 1.5m/hr. Inside the reactor, a selection process occurs which can result in the growth of anaerobic micro-organisms in kind of conglomerate (granules) varying between 0.5-5mg in diameter. These granules are powerful biocatalysts and convert the biodegradable organic matter in the influent in a rapid (space loadings varying from 10-20kg chemical oxygen demand (COD)/m^3/reactor/day) and complete way to biogas. Actually, the granular biomass is such a valuable biocatalyst that it is the only mixed culture which is commercially handled world wide at a respectful price of the order of about 1-2USD per Key dry weight. The sludge is separated from the water and the gas phase by means of an internal settler. Generally effluents approaching discharge standards are thus obtained from waste waters from breweries and soft drink plants, from potato processing plants and from certain paper recycling plants.

For concentrated waste waters, an aerobic treatment has to succeed the anaerobic treatment, yet in a similar and less energy consuming manner. At present, several hundreds of UASB reactors have been installed worldwide, particularly to treat industrial waste water with a COD exceeding 2.0 grams per litre (g/l). They are generally implemented when the waste water is rich in carbohydrates and relatively poor in other contaminants (Allison-Onyechere et al., 2007). For tropical countries, direct anaerobic treatment of sewage has become a reality and several full scale systems are currently operated in Columbia, Brazil and India (Van Haandel and Lettinga, 1994). This is a channel that must be explored further in terms of additional sun-heat input (e.g by means of solar heating systems) and nutrient removal by means of nutrient immobilizing straw biofilter as reported by Avnimelech et al., 1993.

4. Anaerobic digestion of water slurries

The authors have carried out anaerobic digestion of different waste waters / slurries and they are highlighted below;

5. Methodology

Wastes procurement

The wastes used for the anaerobic digestion studies discussed below, which invariably were the wastes to be treated were generally procured or collected from the locality. For instance, the cassava waste waters were collected from local processors of "garri" (a staple food consumed in the eastern part of Nigeria). The palm oil sludge was collected from local processors of palm oil in the community. The cow liquor waste was collected from an abattoir in the locality while the Brewery spent grain and carbonated soft drink sludge were obtained from Nigerian Breweries Ltd and 7UP bottling company (a soft drink manufacturing company) all around the locality. The swine dung were collected from the Veterinary farm in the University of Nigeria, Nsukka.

Waste preparation

Since the primary focus of the different studies were on cheaper sources of energy generation while secondarily obtaining waste effluents that are not only harmless to the environment but expected to boost organic farming/Agriculture, some of the waste waters were pretreated with other solid organic wastes to improve on their biogas production potentials. For instance,

1. In one study, cassava waste water (CW) was co- digested with swine dung (SD) in the ratio of 2:1 while the CW alone and SD alone served as control (Ofoefule et al., 2010).
2. In another study, Abattoir cow liquor (CLW) was combined with brewery spent grain (BS) in the ratio 1:1, cassava waste water (CW) in the ratio 1:3 and carbonated soft drink sludge(CS) in the ratio 3:1 (Uzodinma and Ofoefule, 2008).
3. In yet another study, palm oil sludge (POS) was blended with Agro-industrial wastes like cassava waste water (CW), Brewery spent grain (SG) and carbonated soft drink sludge (SL) in ratios of 1:1, 1:1, and 1:1.2 respectively (Uzodinma et al., 2007a).
4. In yet another study, cassava waste water (CW) was combined with palm oil sludge (POS) in the ratio 2:1, powdered rice husk (RH) 1:2.3 and pig dung (PD) 1:1.5. The nature of the wastes determined their combination ratios (Uzodinma et al., 2007b).

Non- waste materials

Other materials used in the studies generally included; metallic prototype biodigesters/fermenters ranging from 50L capacity (Fig. 1) to 136L capacity (Fig. 2) fabricated locally at the National Centre for Energy Research and development, University of Nigeria, Nsukka. Other materials also used were; Top loading balance (50kg capacity "Five goats" model no Z051599), plastic water troughs, graduated transparent plastic buckets for measuring daily gas production, digital pH meter (Jenway 3510), thermometer (-10-110°C), hosepipes, biogas burner fabricated locally for checking gas flammability.

Fig. 1. 50L Capacity Metallic Prototype Biodigester

Fig. 2. 136L Capacity Metallic Prototype Biodigester

Experimental studies

The wastes were generally mixed with water in the ratio of 2:1 except in the cases where the wastewaters were used alone as control. In such instances, the waste waters were used as they were without further dilution since the constituents were mainly water (93-95%). The digesters were charged up to ¾ level leaving ¼ head space for gas collection. They were stirred thoroughly and on a daily basis throughout the retention period to ensure homogenous blend of the wastes and dispersion of microbes in the entire mixture. Gas production measured as dm^3/kg slurry or L/Total mass of slurry were obtained by downward displacement of water by the gas.

Analyses of wastes

Physicochemical properties of the wastes such as ash, moisture, crude fibre contents, crude fat, crude nitrogen and protein contents, carbon, energy, total and volatile solids were generally determined for all the wastes using recognized laboratory procedures. These properties inherent in the wastes determine and explain the behavior of the wastes during anaerobic digestion. Biochemical analyses such as pH, ambient and influent temperatures were also monitored on the waste slurries as the digestion of the waste progressed. Microbial analysis was also carried out to determine the microbial total viable counts (TVC) for the waste slurries at different periods during the digestion; at the point of charging the digester, at the point of flammability, at the peak of gas production and at the end of the retention period. In some cases flammable gas composition from the different wastes were also analyzed.

6. Results and discussion

The various results obtained during each of the studies are as itemized below:

1. **Anaerobic batch co-digestion of cassava waste water and Swine dung**

The cassava waste water alone had the highest yield of biogas production (130 dm^3/Total mass of slurry) even though the gas produced was not flammable throughout the retention period and therefore does not meet the desired need for cooking and lighting but would however be okay for the purposes of ordinary treatment of the waste water. The non flammability of the gas produced was attributed to the acidic nature of the waste. The microbes that convert wastes to biogas are pH sensitive and survive optimally within the pH range of 6.5-7.5 (Runion, 2009). It was observed that the fresh cassava waste water kills plants in the farm. However when subjected to anaerobic digestion for a period of 30 days it can then be used in the farms as a good organic fertilizer for agriculture. The CW and SD (cassava waste water and swine dung blend) had a lower yield of 120L/total mass slurry; however it commenced flammability on the 10th day. The swine alone had a yield of 123 L/Total mass slurry and commenced flammability on the 6th day. The results showed that the animal waste had a positive effect on the cassava waste water since the CW on its own did not produce flammable gas. There was also attendant reduction in the foul odour of the waste after the digestion showing that the anaerobic digestion killed most of the pathogens responsible for the foul odour. Fig 3 shows the daily biogas production for the period, while Table 1 shows the lag period, cumulative and mean volume of gas productions. The lag period is the period from charging of the digester to onset of gas flammability (Ofoefule et al., 2010).

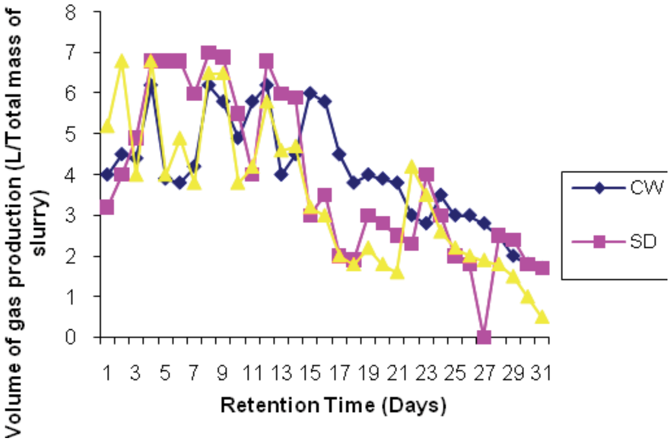

Fig. 3. Daily biogas production

PARAMETERS	CW	SD	CW : SD
Lag period (days).	Nil	5	9
Cumulative gas yield (L/ total mass of slurry).	130.25	122.55	119.90
Mean gas yield (L/ total mass of slurry).	4.20±1.32	3.95±2.01	3.87±1.80

Table 1. Lag period, Cumulative and mean volume of gas production of the pure wastes and blend

2. **Effect of Abattoir cow liquor waste on biogas yield of some Agro-Industrial wastes.**

The results in this study showed that the cow liquor waste and cassava waste water blend (CLW: CW) did not flame throughout the retention period as a result of the acidic nature of the combined waste (pH=3.3). The carbonated soft drink sludge that commenced flammable biogas production on the 9th day stopped after one and half weeks as a result of the drop in pH from 5.68 to 5.20. The reduction in pH killed the microbes responsible for converting the waste to biogas. However the CLW: BS (cow liquor waste: brewery spent grain) had the shortest onset of gas flammability and highest cumulative gas yield of 613.2 L/TMS (Table 2). Fig 4 shows the daily biogas production (Uzodinma and Ofoefule, 2008).

Parameters	**BS**	**CS**	**CW**	**CLW : BS**	**CLW: CW**	**CLW: CS**
Lag period (days)	20	8	Nil	6	9	8
Cumulative gas yield (L/TMS)	183.6	177.50	Nil	613.2	394.2	87.4
Mean Volume of gas yield (L/TMS)	7.34	7.10	Nil	24.53	8.23	2.54

Table 2. Lag periods, cumulative and mean volume of gas yield for single organic wastes and CLW blends

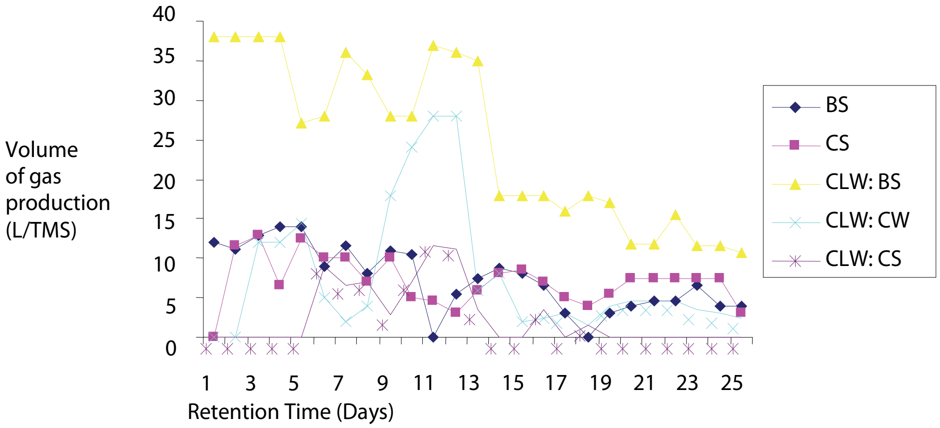

Fig. 4. Daily biogas yield

3. Preliminary studies on biogas production from blends of palm oil sludge with some Agro-based wastes.

The palm oil sludge (POS) in this study could not produce quantifiable gas within the 25 days retention period used for the experiment. However when combined with brewery spent grain (SG), carbonated soft drink sludge (SL) and cassava waste water (CW), reasonable quantities of biogas were produced which flamed after some lag periods as shown in Table 3. The POS: CW had the highest yield of biogas followed by the POS: SG while the least yield came from the POS:SL. The better yield of POS: CW over the others could be accounted for by the fact that the CW and others were allowed to be partially decomposed for a period of two months to increase their pH level, since in their fresh state they were found to be acidic. This resulted in the cassava waste water giving a better yield of biogas. Analysis of their flammable gas composition showed that POS: CW and POS: SL gave higher methane contents than POS: SG (Table 4). Fig. 5 shows the Daily biogas production (Uzodinma et al., 2007a).

Parameters	**POS:SG**	**POSL:CW**	**POS:SL**
Lag period (days)	10	8	15
Cumulative gas yield (L)	312	394.2	87.4
Mean volume of gas yield (L)	12.5	15.8	3.5

Table 3. lag periods, cumulative and mean volume of gas yield for POS blends

Waste blends	CO_2	CO	H_2S	CH_4
POS:SG	25.3	5.0	2.5	67.2
POS:CW	20.9	1.6	1.3	76.2
POS:SL	20.1	1.2	2.2	76.5

Table 4. Analysis of flammable gas composition for POS blends (%)

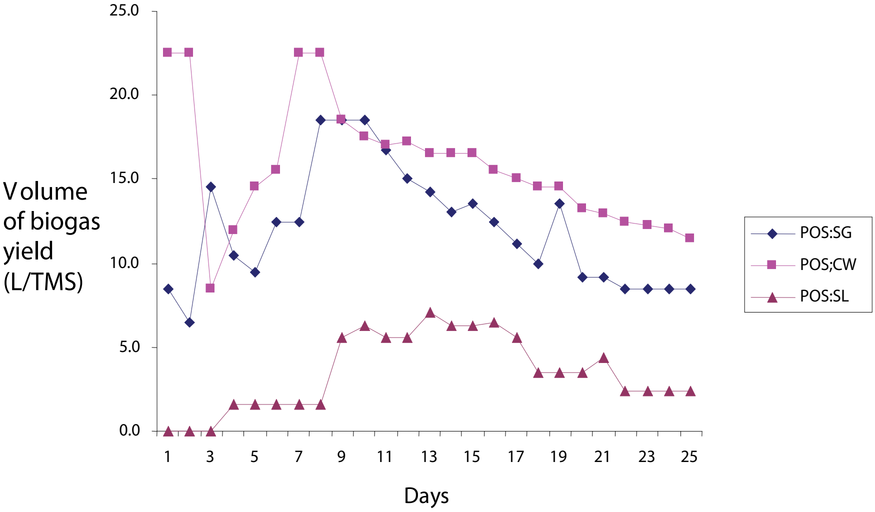

Fig. 5. Daily biogas yield for POS blends

4. **Energy generation from microbial conversion of Treated cassava waste water from garri processing industry.**

In this study, cassava waste water (CW) was treated with some other wastes to improve its pH level before digesting it. The waste used included; palm oil sludge (POS), powdered rice husk (RH) and pig dung (PD). The results showed that not only was the pH increased, the physicochemical properties also improved, which translated to higher biogas yields. The CW: RH gave the highest yield while the CW: PD followed with the shortest lag period of 4 days (Table 5). The higher yield of CW: RH was attributed to the fact that the rice husk was pre-decayed for about 1 month, and as a result had accumulated some microbes that aided in the faster digestion. The shortest lag period of CW: PD was explained by the fact that swine dung is a rumen animal, having the natural flora that are responsible for biogas production in its gut, aiding the fastest onset of gas flammability. Fig. 6 shows the Daily biogas production (Uzodinma et al., 2007b).

Parameters	CW:POS	CW:RH	CW:PD
Lag period (Days	8	6	4
Cumulative volume of gas Production (L/TMS)	394.20	481.30	432.00
Mean volume of gas production (L/TMS)	15.77	19.30	17.30

Table 5. Lag Period, Cumulative and Mean volume of biogas production

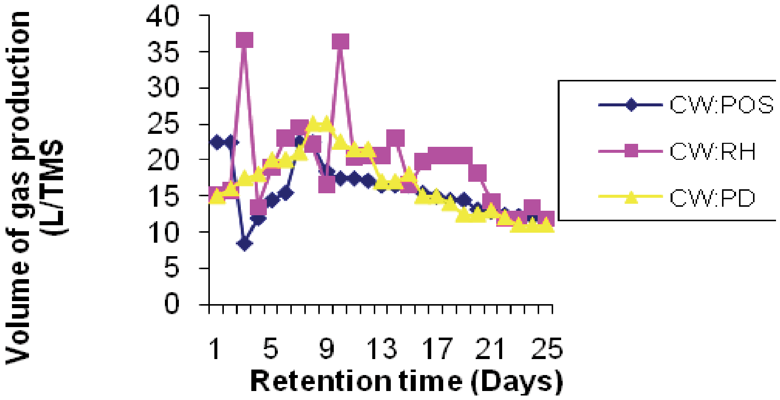

Fig. 6. Daily biogas production

Socio-Economic Benefits of Waste Water Treatment

Apart from reduction in environmental pollution from the treatment of waste waters, new demands for agricultural products arising from increased biomass usage would impact on the social-economic life of the populace especially when anaerobic digestion process of waste water treatment option is undertaken. Social issues such as employment generation, and poverty reduction especially for the developing countries would be addressed through this technology as a result of expanded economic activities across the real sector of the economy encompassing agriculture, manufacturing and exports. These would enhance people's ability to develop economic activities designated to reduce poverty particularly for the rural communities. Conversion of these biodegradable waste waters (both domestic and industrial) into biogas would result in cleaner air as well as efficient waste management system, improving the sanitary conditions of the urban environment. This will lead to socio-economic benefits with regard to health, income and security of the eco-system threatened by adverse climatic alterations (Ofoefule et al., 2009).

5. Conclusion

The results of these studies have shown that the waste waters/ slurries which are pollutants in the areas where they are processed can be sources of useful energy and organic fertilizers by subjecting them to anaerobic digestion for biogas production. The studies further revealed that most of these waste waters on their own are not capable of effective and efficient biogas production since they are mostly found to be acidic in their fresh states. They therefore need to be co-digested with other better producing wastes like animal wastes to enhance their flammable biogas production capabilities. The anaerobic digestion process of these waste waters is expected to be a source of waste management and pollution control.

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Agricultural Dairy Wastewaters

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1. Introduction

In Ireland, farming is an important national industry that involves approximately 270,000 people, 6.191 million cattle, 4.257 million sheep, 1.678 million pigs and 10.7 million poultry (CSO, 2006). Agriculture utilizes 64% of Ireland's land area (Fingleton and Cushion, 1999), of which 91% is devoted to grass, silage and hay, and rough grazing (DAFF, 2003). Grass-based rearing of cattle and sheep dominates the industry (EPA, 2004). Livestock production is associated with external inputs of nutrients. Phosphorus (P) surpluses accumulate in the soil (Culleton et al., 2000) and contribute to P loss to surface and groundwater (Tunney, 1990; Regan et al., 2010). Elevated soil P status has been identified as one of the dominant P pressures in Ireland (Tunney et al., 2000). Schulte et al. (2010) showed that it may take many years for elevated soil P concentrations to be reduced to agronomically and environmentally optimum levels. The extent of these delays was predominantly related to the relative annual P-balance (P balance relative to total P reserves). While the onset of reductions in excessive soil P levels may be observed within five years, this reduction is a slow process and may take years to decades to be completed.

Agricultural wastes and in particular dairy slurry and dirty water are discussed in this chapter. However, while the term 'waste' is commonly used for these materials, it is an unfortunate label, as it suggests that the materials have no further use and are merely a nuisance by-product of farming systems that must be managed. However, given the high nutrient contents of these materials, it is far more appropriate for them to be considered as organic fertilizers, and as such being a valuable commodity for the farmer. With higher and more volatile chemical fertilizer prices in recent years, the fertilizer replacement value in economic terms of these materials is increasing. Therefore, the management of agricultural 'wastes' in a manner that maximises the nutrient recovery and fertilizer value to crops should be a priority within any management plan for these materials.

Nutrient contents and various research areas regarding management, remediation and control of such nutrients to prevent losses to the environment are discussed. The Surface Water Directive, 75/440/EEC (EEC, 1975), the Groundwater Directive, 80/68/EEC (EEC, 1980), the Drinking Water Directive, 98/83/EC (EC, 1998), the Nitrates Directive, 91/676/EEC (EEC, 1991(a)) and the Urban Wastewater Directive, 91/271/EEC (EEC, 1991(b)), combined with recent proceedings taken against the Irish State by the EU Commission alleging non-implementation of some aspects of the directives, has focused considerable attention on the environmentally-safe disposal of agricultural wastewaters in Ireland. To address these directives, the WFD (2000/60/EC, 2000) came into force on 22nd December, 2000 and was transposed into Irish legislation by the European Communities (Water Policy) Regulations 2003 on the 22nd December, 2003. Eight "River Basin Districts" (RBD) were established in Ireland, north and south, with the aim of achieving "good status" in all surface and groundwater by 2015. The WFD will bring about major changes in the regulation and management of Europe's water resources. Major changes include:

- A requirement for the preparation of integrated catchment management plans, with remits extending over point and non-point pollution, water abstraction and land use;
- The introduction of an EU-wide target of "good ecological status" for all surface and groundwater, except where exemptions for "heavily-modified" water bodies are granted. Programmes of measures (POM) must be put in place to protect groundwater and surface water while being efficient and cost-effective. POM to achieve at least "good ecological status" must be implemented by the agricultural sector by 2012. In Ireland the Nitrates Directive is the main POM in place. At present, a strategy exists within Europe to restore the "good ecological status" of surface and groundwater. It focuses on reducing nutrient pressures to prevent further nutrient loss to surface and groundwater. However, intensification of agriculture poses a challenge to the sustainable management of soils, water resources, and biodiversity. N losses from agricultural areas can contribute to ground- and surface water pollution (Stark and Richards, 2008; Humphreys et al., 2008).

Results from a Water4all project suggest that regulation alone will not achieve sufficient reduction in water quality as nitrate builds up in soils and the long residence time of groundwater in aquifers needs a more immediate solution (Water4all, 2005; Hiscock et al., 2007). Therefore, remediation (nitrogen - N) and control (phosphorus – P) technologies must be an integral part of the process for point and diffuse pollution from historic or future incidental nutrient losses. Solutions developed must be integrated efforts within a catchment or river basin.

Good Agricultural Practice Regulations under The Nitrates Directive (European Council, 1991) is currently the main mitigation measure in place within the agricultural sector to achieve the goals of the WFD. These regulations came into effect in the Republic of Ireland in 2006 under Statutory Instrument (S.I) 788 of 2005, and subsequently under S.I 378 of 2006, S.I 101 of 2009 and S.I 610 of 2010. The Nitrates Directive sets limits on stocking rates on farms in terms of the quantity of N from livestock manure that can be applied mechanically or directly deposited by grazing livestock on agricultural land. A limit of 170 kg N ha^{-1} year^{-1} from livestock manure was set. However, the EU Nitrates Committee approved Ireland's application for a derogation of this limit to allow grassland-based (mostly dairy) farmers to operate at up to 250 kg N ha^{-1} year^{-1} from livestock manures, with the understanding that this derogation will not impinge on meeting the requirements of the Nitrates Directive. The current average stocking density on dairy farms is 1.81 livestock units (LU) ha^{-1}.

The "Good Agricultural Practice for the Protection of Waters" regulation, S.I 778 of 2005 (Anon, 2005), came into effect on February 1st 2006. The most recent revision of the regulation was published in 2010 (Anon, 2010). It constrains the use of P and N fertilizers, ploughing periods and supports derogation on livestock intensity. In particular it regulates farmyard and nutrient management, but also examines prevention of water pollution from fertilizers and certain activities. The linkage between source and pathway can be broken if pollutants remain within farm boundaries and are not discharging to drainage channels, subsurface drainage systems, or entering streams or open waterways within farm boundaries. These regulations also place restrictions on land spreading of agricultural wastes. This strategy looks at present loss and future loss prevention. There are no guidelines in place for the remediation or control of contaminated discharges to surface and/or groundwater or future discharges due to incidental losses. Traditionally, agricultural wastes are managed by land spreading. Following land spreading, the recharge rate, the time of year of application, the hydraulic conductivity of the soil, the depth of soil to the water table and/or bedrock, and the concentration of nutrients and suspended sediment in the wastewater (dirty water and any discharge containing nutrients) are some of the defining parameters that determine nitrate movement through the soil to the watertable. The maximum instantaneous rate of application is 5 mm per hour and the quantity applied should not exceed 50 m^3 per hectare per application (ADAS, 1985; 1994; DAFF, 1996) and these recommendations are present within best farm management practices. Infiltration depth of irrigated water and rainfall may be estimated when the annual effective drainage, number of effective drainage days, effective porosity, annual precipitation, and the hydraulic load of the irrigator are known (Fenton et al., 2009(b)). This data may then be combined with watertable data to examine if excess nutrients recharge to groundwater within a specific time frame.

2. Agricultural dairy wastes

2.1 Types of dairy wastes and nutrient content

In a grassland system, the N recovery rate of dairy slurry is highly variable due to variations in slurry composition, application methods, spreading rates, soil and climatic conditions and slurry N mineralisation rates (Schröder, 2005). In Ireland, approximately 80% of manures produced in winter are managed as slurries containing 70 g kg^{-1} dry matter, 3.6 g kg^{-1} total N (TN) and 0.6 g kg^{-1} total P (TP) (Lalor et al., 2010(a)). About 50% of the TN is in ammoniacal form and has the potential to be volatilised as ammonia during storage and following land spreading. Estimated organic managed waste generation for Ireland is presented in Table 1.

Waste Category	Waste Generation	
	Tonnes wet weight	%
Cattle manure and slurry	36,443,603	60.6
Water (dairy only)	18,377,550	30.5
Pig slurry	2,431,819	4.0
Silage effluent	1,139,231	1.9
Poultry litter	172,435	0.3
Sheep manure	1,336,336	2.2
Spent mushroom compost	274,050	0.5
Total	60,170,025	

Table 1. Estimated agricultural organic managed waste generation in 2001 (EPA, 2004a).

Great variation in the nutrient content of dairy slurry exists depending on feed type, age of sample when tested, age of the animal and how the effluent is stored and managed (Smith and Chambers, 1993). Seasonal differences in nutrient contents also exist (Demanet et al., 1999). Tables of published slurry nutrient contents in Europe exist (see MAFF, 2000). Such values are similar to South American dairy slurry concentrations found by Salazar et al. (2007). Some dairy slurry concentrations for undigested and digested samples are presented in Table 2. These tend to be similar to other nutrient contents across Europe found by Villar et al. (1979); Scotford et al. (1998(ab)) and Provolo and Martínez-Suller (2007). In Ireland, dirty water is generated from dairy parlour water and machine washings, precipitation and water from concreted holding yards (Photo 1). Average dirty water production per cow is 49 L^{-1} day^{-1}. Although dilute, dirty water has sufficient nutrients to give rise to eutrophication if lost to a waterbody through runoff or excess infiltration. Implementation of current legislation requires separation of faecal matter and water, thus diminishing the nutrient content of dirty water for land application (Photo 1). As the nutrient content is reduced and storage and water charges are high, an alternative solution to dirty water management is remediation and re-use for washing yards (Fenton et al., 2009). A number of papers have reported the chemical composition of dirty water from dairy farms (ADAS, 1994; Cumby, 1999; Ryan, 2006; Fenton et al., 2009(a);Minogue et al., 2010). Table 3 presents a range of nutrient contents available in dirty water from a number of studies. Minogue et al. (2010) and Cumby (1999) report higher mean TN nutrient figures for 20 farms in England and Wales of 580±487 mg TN L^{-1}. Martínez-Suller et al. (2010(b)) reviewed the composition of dirty water in the literature including others not mentioned in Table 3.

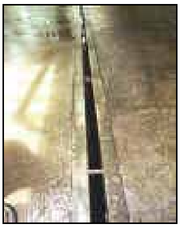

Photo 1. Dirty water generation: wash down high volume low pressure hose and drainage channel for speeding up washing after milking (Source: www.teagasc.ie)

Prediction of the nutrient content of agricultural waste waters would help farmers to more accurately calculate the nutrient fertiliser replacement value of the landspread materials and the additional fertiliser requirements for their crops. Martínez-Suller et al. (2010(a)) suggest that dry matter content or electrical conductivity are rapid, cheap methods to estimate the nutrient content of waste waters and manures.

2.2 Faecal microorganisms

Agricultural wastes not only pose a threat to waterbodies, a second major concern is the presence of pathogenic and/or antibiotic resistant bacteria in animal wastes (Sapkota et al., 2007) and the threat to human health. If properly handled and treated, manure is an effective and safe fertiliser. However, if untreated or improperly treated, manure may become a source of pathogens that may contaminate soil, food-stuffs, and water bodies (Vanotti et al., 2007). Animal manures are known to contain pathogenic bacteria, viruses and parasites (Pell, 1997). The contamination of surface waters with pathogenic micro-organisms transported from fields to which livestock slurries and manure have been applied is a serious environmental concern as it may lead to humans being exposed to such micro-organisms via drinking water (Skerrett and Holland, 2000); bathing waters (Baudart et al., 2000); and water used for the irrigation of ready to eat foods (Tyrel, 1999). A recent study

Study	Manure Type	Digested/ Undigested	Nh_4-N	Organic N	NO_3-N	TN	TP
			mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
Mulbry and Wilkie, 2001	Dairy	Undigested	306	904	<1	1210	303
		Digested (Maryland, USA)	1620	751	<1	2371	240
		Digested (Florida, USA)	178	47	<1	225	24.7
Pizarro et al., 2002	Dairy	Digested	5 to 80	-	-	-	1 to 20
Wang et al., 2009	Dairy	Digested (Minnesota)	2232			3456	249.7
	Dairy	Undigested (Minnesota)	1782			3305	266
Kebede-Westhead et al., 2004	Dairy	Digested manure effluent (Florida, USA)	233		<1	412	64.5
Brennan et al., 2010(abc)	Dairy	Undigested (West Ireland) May	1759(123)			4433 (271)	1138 (76)
	Dairy	Undigested (West Ireland) July				4861(425)	1144 (93)
	Dairy	Undigested (West Ireland) December				3982(274)	811(37)
Martínez -Suller et al., 2010	Dairy	Undigested (South East Ireland)				3430(1400)	560(250)
() standard deviation							

Table 2. Dairy slurry nutrient content from various studies.

Study	Number of farms	Period of study		BOD_5	K	NH_4-N	P	TP	TN
Minogue et al., 2010	60-Ireland	Monthly for 1 year	Mean	2246	568	212	37	80	587
			Min	0	3	0	0	2	0
			Max	19085	7232	2933	1240	795	6030
			SD	2112	513	206	53	68	536
Martínez-Suller et al., 2010(b)	1-Ireland	13 weeks (January – May)	Mean	3084	415	32	8.5	44	351
			Min	1570	213	0	0.7	21	128
			Max	8400	977	106	24.9	103	987
			SD	1739	169	25	6.49	22	231
Serrenho et al., 2010	1-Ireland								
Fenton et al., 2009(a)	1-Ireland	3 months (August - October)	Mean	-	-	-	14.0(9.2)	20.1 (6.9)	170(33.2)
Singh et al., 2005				3000				111	479
Dunne et al., 2005(ab)	1-Ireland	Winter		2828		42			
		Spring		2703		53			
		Summer		2682		36			
		Autumn		2303		6			
Ryan et al., 2005	1-Ireland		Min	-	-	-	-	-	43
			Max	-	-	-	-	-	126
Rodgers et al., 2003	1-Ireland			2208					
Cannon et al., 2000				1440					
Cumby et al., 1999	20- England and Wales	Summer		9670	150	58	-	49	95
		Autumn		7450	85	48	-	34	70
Richards, 1999	1-Ireland					84.4			188
Misselbrook et al., 1995	1-England				350	42		<100	450
Ryan, 1991	1-Ireland			2077	210	92	17	23	159

Table 3. Dairy dirty water nutrient results from various studies in U.K. and Ireland. Based on new data and Martínez-Suller et al., 2010(b). All units in mg L^{-1}

(Venglovsky et al., 2009) has shown that animal manure contributes significantly to pathogen loading of soil and consequently runoff to waterways. Furthermore, a recent report by the EPA in Ireland (Lucey, 2009) highlighted land-spreading of manure or slurry as one of the main sources of microbial pathogens in groundwater. Additionally, a report by the Food Safety Authority of Ireland (FSAI, 2008) stated that 'there is potential for the transfer of pathogens to food and water as a result of land-spreading of organic agricultural material'.

Research from New Zealand, shows that dirty water contains faecal micro-organisms, which originate from dairy cattle excreta. Researchers such as Aislabie et al. (2001); McLeod et al. (2003) and Donnison and Ross (2003) have shown transfer of bacterial indicators, faecal coliforms and *Campylobacter jejuni* through soil. The Pathogen Transmission Routes Research Programme in New Zealand showed that significant faeces contamination arose through the deposition of faeces by grazing animals with access to waterways. Fencing and implementation of buffer strips were recommended as mitigation measures to prevent such losses (Collins et al., 2007). Presence of faecal indicator organisms is used to identify waters impacted by faecal matter from mammals. Indicators of faecal contamination such as *E. coli* are widely used as they are faecally specific and believed to not survive for more than 4 months post excretion (Jamieson et al., 2002). Recent research has shown that *E. coli* can survive for long periods of time in temperate soils (Brennan et al., 2010) and contribute to high detections in drainage waters from agricultural soils. *E. coli* were particularly associated with poorly drained soils due to the greater persistence of preferential flow channels and anaerobic micro-sites where they might survive. Thus the presence of *E. coli* in waters may not indicate recent contamination by faecal matter but could be due to historical pathogen deposition. Many treatment systems may be used to treat livestock waste and remove or decrease viral, bacterial and eukaryotic pathogens. Examples include bio-gas producing anaerobic digestion, composting, aeration, storage under a variety of redox conditions, and anoxic lagoons, all of which have been reviewed by Topp et al. (2009).

2.3 Current management practices for agricultural waste waters

The Nitrates Directive and rising costs are now forcing better use of nutrients in slurry. Research in the U.K. (Misselbrook et al., 1996; 2002; Smith and Chambers, 1993; Smith et al., 2000) includes improving N recovery from slurry by examining the effect of spreading method and timing, and reducing ammonia (NH_3) losses from slurry by evaluating splash-plate versus alternative techniques such as trailing shoe or trailing hose slurry application methods. The average abatement of these methods varies and differs when grassland or arable application are considered (Smith and Misselbrook, 2000; Misselbrook et al., 2002). Present research in Ireland follows similar patterns (Ryan, 2005). Ammonia emissions with respect to trailing shoe versus splash-plate and subsequent N uptake by the sward are being investigated in Irish grasslands (Lalor and Schulte, 2008). Farm management strategies aimed at prevention of nutrient loss to water have recently been reviewed by Schulte (2006).

The Nitrates Directive regulations impose limits to N and P inputs onto livestock and tillage farms. Cattle and dairy farming systems are required to make more efficient use of nutrients. International experience suggests that significant gains in nutrient efficiency can be made by increasing the utilisation of N in slurry. Lalor (2010(b)) suggested N-utilisation efficiencies from slurry as low as 5% under existing practices, whereas international literature suggests that there is scope to raise efficiencies to 40-80%. Despite the relatively low utilisation in practice, the Nitrates regulations set a nitrogen fertilizer replacement value

(NFRV) target of 40%, presenting a considerable challenge for the grassland sector. In addition, the ceiling to nutrient inputs imposed under the Nitrates Directives made it difficult for many livestock farmers to continue to accept pig slurry as a fertilizer onto their farm. In Ireland, as a result, the potential for the traditional practice of spreading slurry on grasslands has been reduced significantly. Returning pig slurry to arable land allows a more closed nutrient cycle to operate, since cereal grains constitute a significant proportion of the diet of pigs. However, this creates a major logistic challenge where arable land and pig farms are not closely located (Lalor et al., 2010(b)).

In an Irish study, cattle slurry application on grassland shows that the NFRV in the year of application is affected by application method and timing. Cattle slurry applied (using traditional methods) with splashplate had an NFRV of 21% in April and 12 % in June. Application using trailing shoe (a modern alternative which places slurry in thin bands along the soil surface) increased the NFRV to 30% in April and 22% in June. Changing application timing from summer to spring with existing splashplate machinery is the most cost effective strategy for improving NFRV. Approximately 4% of the total slurry N applied was recovered in the second year after application. For repeated applications over a number of years, models indicate that the maximum cumulative residual recovery would be 12-14% of the annual slurry N application rate. It would take approximately 10 years of repeated slurry applications for the residual N release to reach this maximum level (Lalor et al., 2010(b)). In Ireland, research by Lalor et al. (2010(b)) showed that the NFRV target of 40% set in the Nitrates regulations can only be achieved when the residual N release is included, and when best practice strategy of trailing shoe application in April was adopted. Spring application of slurry is often restricted by soil trafficability, particularly on poorly drained soils. The trailing shoe application method can provide more flexibility for spring application as grass contamination is reduced compared to splashplate.

In Ireland, besides land application methods (splashplate or trailing shoe (Photo 3)), dirty water irrigation using centre pivotal irrigation systems is common (Photo 2). The recommended irrigation rates should not exceed 5 mm hr^{-1}. Strict guidelines for their safe use are in place. Application timing of dirty water should take soil moisture status and soil physical properties into account (Houlbrooke et al., 2004). Two pond systems are used in many countries reducing the biological oxygen demand and suspended solids contents. A limitation here is that the nutrients remain unchanged and need to be landspread with potential environmental consequences. An upgraded “advanced pond system” has been

Photo 2. Rotational centre pivot sprinkler system used for dairy dirty water irrigation (Source: www.teagasc.ie)

Photo 3. Slurry tanker with trailing shoe application system (Source: Teagasc)

designed by Craggs et al. (2004) and could be an alternative on dairy farms. Houlbrooke et al. (2006) showed that individual irrigation systems with low intermittent irrigation rates (0.4 to 4 mm hr^{-1}) could be used without nutrient losses. To facilitate this low irrigation rate, increased storage is needed on a farm. Adapted low irrigation lines have now also been investigated, the position of which may be changed through use of a quad-bike system.

Bolan and Swain (2004) reviewed issues and innovations in land application of farm wastes in New Zealand and showed that research must focus on improved systems to convert manure based wastes into a valuable but also environmentally benign product.

An alternative manure management system in some countries is anaerobic digestion. Manures are an excellent source of organic material for anaerobic digestion and the production of bio-gas. Co-digestion of agricultural wastes with sewage sludges can further improve the methane production in anaerobic digesters (Ward et al., 2008).

2.4 Environmental Impact of agricultural waste waters

Agricultural waste waters can contain N, P, K, S, C, pathogenic micro-organisms and a range of other micro nutrients. Nutrients returned to agricultural soils through land-spreading are important for nutrient efficiency on farms and for reducing reliance on inorganic fertilisers. Land application should be at rates that supply nutrients for crop growth and at the time when these nutrients are required. Addition of excessive nutrients at times of reduced crop demand can increase the potential for losses of nutrients such as N and P, which contribute to surface water eutrophication and can lead to pollution of drinking waters. In addition, land application to wet soils can lead to increased emissions of greenhouse gases such as nitrous oxide (N_2O).

Landspreading of dairy slurry and wastewaters has been associated with ammonia volatilisation to the atmosphere. Application of ammoniacal nitrogen (NH_4^+) to soils in wastewater increases the soil solution NH_4^+ concentrations, which is in equilibrium with free ammonia (NH_3) which is also in equilibrium with the concentrations in the atmosphere (See Equation 1).

$$\begin{tikzcd} \text{Atmosphere:} & NH_3 \arrow[d, leftrightarrow] \\ \text{Soil Solution: } NH_4^+ + OH^- \arrow[r, leftrightarrow] & NH_3 + H_2O \end{tikzcd}$$

Ammonia volatilisation from soil lowers the pH of soil directly under the waste water. Further soil pH reduction can also occur when the volatilised NH_3 is re-deposited and nitrified. Agriculture is the main emitter of NH_3 to the atmosphere accounting for ~80% of total global emissions (Stark and Richards, 2008) and is expected to reach 109 Tg N yr^{-1} by 2050. Once in the atmosphere, NH_3 can readily combine with NO_3 and SO_4^{2-} in acid cloud droplets to form particulates and can be transported over long distances before being deposited again to soil or water. Atmospheric N deposition has increased over recent decades and ranges from 5 to 80 kg ha^{-1} yr^{-1} with a global average of 17 kg ha^{-1} yr^{-1} have been observed. Deposited NH_3 can then lead to acidification of soil and eutrophication of waters, which led to the UN establishing the Convention on Long-range Transboundary Air Pollution including NH_3 and the EU set limits for NH_3 from European countries. Emissions of NH_3 from agricultural slurry and waste waters can be reduced through utilisation of low emission storage facilities where stores are covered to reduce contact with the atmosphere. Emissions can also be reduced from the field through the use of low emissions spreading methods such as band spreading and injection (See section 2.3). Land application of dilute waste wasters has lower NH_3 emissions compared to more solid waste due to a reduction in the NH_4^+ content and the infiltration of the liquid waste into soil, reducing atmospheric contact. Thus dilute effluents have lower NH_3 emissions, but potentially greater N_2O and NO_3^- emissions.

Application of animal slurries and wastewaters to soils promotes denitrification through the supply of readily available N and C for microbial respiration and also by promoting anaerobic conditions in the soil through partial sealing of soil pores and the consumption of oxygen through C oxidation. Storage of manures leads to the build up of volatile fatty acids which are readily degradable forms of C. Microbial denitrification associated with landspreading of organic wastes can be an important source of the potent greenhouse gas, N_2O. Emissions of N_2O from slurry spreading are mainly related to the application method, and the soil temperature combined with soil moisture at the time of application. Methods for reducing N_2O emissions associated with waste waters include limiting the hydraulic loading to ensure soils remain aerobic; adjusting application timing to when soils are not anaerobic; adjusting application method/rate; inclusion of nitrification inhibitors to slow the rate of NO_3^- formation; manipulation of the C/N ratio; digestion or storage to reduce labile C content and inclusion of materials with high cation exchange capacity e.g. zeolite. A schematic of soil N transformations is presented in Figure 1.

There have been numerous reports of water pollution occurring after landspreading of wastewaters to soils. Richards et al. (2004) reported nitrate leaching losses ranging from 95 to 323 kg N ha^{-1} when wastewaters were over-applied to free draining soils. Houlbrouke et al (2004) reported between 2 and 20% of N and P applied in agricultural wastewaters leached through soils and the concentrations leaching were above ecological limits for good water quality.

Repeated application of wastewaters to soil can lead to an increase in the organic fractions of N, P, K and organic carbon due to changes in soil organic matter. In New Zealand, Barkle et al. (2000) reported significant increases in soil total N and organic C. At low temperatures increasing soil C content due to dirty water application can lead to greater N immobilisation due to changes in the soil C/N ratio (Ghani et al., 2005). Increasing soil nutrient status above the agronomic optimum has been shown to increase the risk of nutrient loss to water (Sharpley and Tunney, 2000). Other soil properties can be influenced by land application such as increasing soil pH, changes in soil hydraulic conductivity due to clogging, plugging and macropore/aggregate collapse. Often the actual effect of landspreading on soil physical properties is difficult to quantify due to variability in soil physical properties, short term observation and experimental approaches within a background of seasonal variation in properties (Hawke and Summers, 2006) Agricultural waste waters can contain high concentrations of pathogen micro-organisms such as Campylobacter, Listeria, Cryptosporidium and Salmonella spp. Loss of high concentrations of faecal pathogens to water can result in the waters being unfit for human consumption and failing to meet water quality standards for bathing water quality. Pathogen transfer to water can occur when waste waters are applied to water-logged soils where water flow over soil leads to high pathogen losses to rivers and associated bathing waters (Kay et al., 2007). Reducing the volume and area contaminated by waste waters on farms can reduce emission of pathogens to water by 10% (Kay et al., 2007).

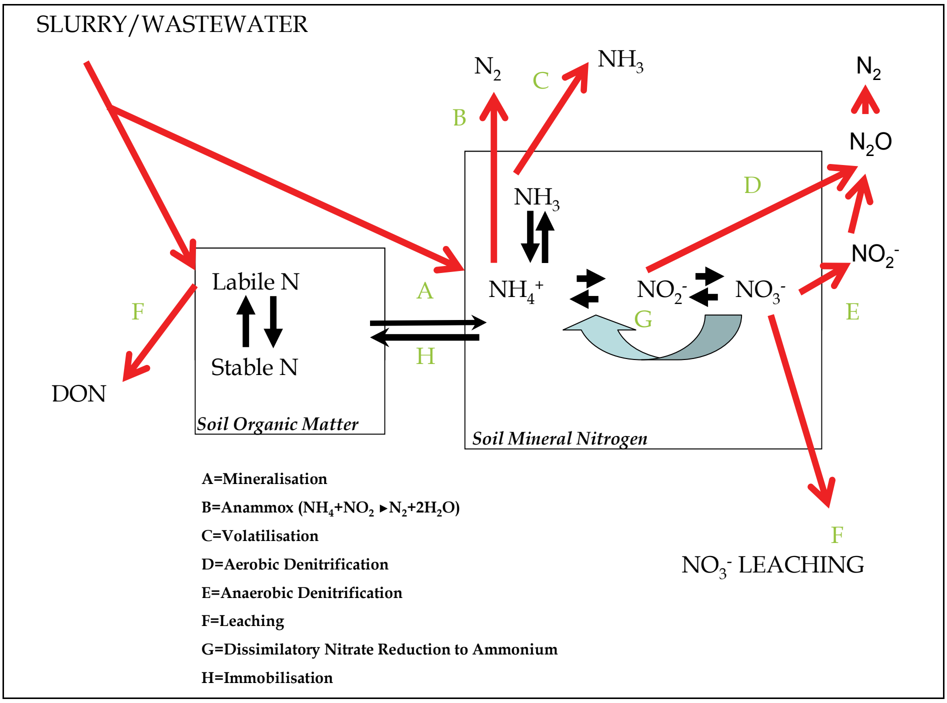

Fig. 1. Soil N transformations of slurry/wastewater derived nitrogen inputs.

3. Novel remediation techniques currently being researched

Fenton et al. (2008) reviewed agricultural wastewater remediation and control technologies suitable for Ireland. Several options such as use of chemical amendments, subsurface carbon emplacement and wetlands were some of the options proposed for further research.

3.1 Amendments to dairy slurry and dirty water

Dairy dirty water is a bio-product of dairy farming. The usual method for disposal of this product is land-spreading (Healy et al., 2007). This can increase the P concentration on the soil surface and the pollution related with the natural run-off during rain events. Not many studies have been made regarding this subject.

Due to the properties of the dairy dirty water, the potential for leaching should also be considered. Usually, P leaching is not considered to be a significant problem in groundwater because it is not very mobile in soils or sediments, and should therefore be retained in the soil zone. However, in extremely vulnerable areas, where the soil and subsoil are shallow and where P enters groundwater in significant quantities, groundwater may act as an additional nutrient enrichment pathway for receptors such as lakes, rivers and wetlands (EPA, 2008). Phosphorus leaching may occur in sandy soils (Carlyle et al., 1998) or where there are preferential flow paths in the soil.

In the past, the primary objective of chemical amendment of manure was to reduce NH_3 losses from manure as this increased N availability to plants. In recent years environmental concerns have shifted this focus to amendments, which mitigate P loss from soils and manure. In Ireland, the focus of recent research has been to find amendments which reduce solubility of P in dairy cattle slurry in particular. The use of such amendments must be practical and cost effective for the farmer. The effect of reducing P solubility on reducing subsequent P fertilizer replacement value of the material should also be considered.

Alum (aluminium sulphate) has been used extensively to treat poultry litter in the U.S for over 30 years with great success to reduce NH_3 in poultry houses and reduce soluble P in poultry litter (Moore and Edwards, 2007). These authors also found that alum addition to poultry litter reduced P loss, ammonia volatilisation and had negligible effect on metal release from amended soil. Work involving amendments of swine and dairy cattle slurries for the control of P have been limited to laboratory batch studies with little emphasis on cost or feasibility of treatments (Dao, 1999; Dou et al., 2003; Kalbasi and Karthikeyan, 2004; Smith et al., 2001; Moore et al., 1998).

Aluminium chloride has been recommended as the most suitable amendment for controlling P solubility in swine and cattle slurry (Smith et al., 2001). In an incubation study Dou et al. (2003) found that technical grade alum added at 0.1 kg/kg (kg alum per kg slurry) and 0.25 kg/kg reduced Water Extractible P in dairy and swine slurry by 99% and 80%, respectively. Dao (1999) amended farm yard manure with calcium carbonate, alum and fly ash in an incubation experiment and reported WEP reductions in amended manure compared to the control of 21, 60 and 85%. Penn et al. (2009) examined the sorption and retention mechanisms of several P sorbing materials (PSMs) including acid mine drainage treatment residuals, water treatment residuals, fly ash, bauxite mining residual and FGD in lab experiments and found the degree of sorption of P to be strongly influenced by the solution pH, buffer capacity of manure, and ionic strength of amendments. These amendments are attractive as they are free. However, they are more variable than chemicals and commercial coagulants used by other workers and much more research is required before there could be used in practice. Internationally, P sorbing amendments have been used to control P losses after manure application. P sorbing amendments can either be added directly to the manure before land application (Moore et al., 1998), spread on the ground before manure application (McFarland et al., 2003), or incorporated into the topsoil at (Novak and Watts, 2005).

Ochre from coal mining origins in the U.K. is a low value waste product from acid mine drainage and has been used as an amendment to sequester P in filters or drainage ditches, or in wetlands receiving sewage or agricultural waste. In Ireland, metal release from metal mining Avoca ochre has made it unsuitable for environmental purpose (Fenton et al., 2008; Fenton et al., 2009(a)). Ochre has a high P sequestration capacity with 97% of sequestration occurring within 5 minutes of contact with an agricultural waste.

3.2 PRB and reactive media for enhanced denitrification

Low-cost, in situ treatment systems, called permeable reactive barriers (PRB), may be used to treat groundwater. In these systems, N-rich wastewater flows through a carbon (C)-rich mixture to reduce nitrate concentrations to acceptable levels. Organic C amendments offer low-cost surface and subsurface treatment alternatives for wastewater treatment. C availability is an important factor that affects denitrifying activity in soils. The presence of C provides an energy source, thereby enhancing the potential for denitrification. Denitrification may be increased in soils by the addition of an external C amendment. This amendment may be natural C such as woodchip, wheat straw, corn, vegetable oil, sawdust mulch, or other materials, such as treated newspaper or unprocessed cotton (Volokita, 1996). A PRB or denitrification wall is only one of many denitrifying bioreactor types, i.e. denitrification beds, up-flow bioreactors, stream bed bioreactor or denitrification layers. The limitations of a denitrification wall are that they require site specific analyses of hydraulic gradient, the depth and extent of the nitrate plume/s, removal of nitrate is confined to up-gradient pollution sources and within the upper 2 m of groundwater. Problems may arise if the denitrification wall has a lower saturated hydraulic conductivity than the surrounding sub-soil. If this occurs, nitrate plumes tend to flow around the wall and not through it. However, in cases where nitrate contamination occurs below 2 m, the diameter (parallel to flow path of contaminant) of the trench may be widened. This causes up-welling into the more permeable trench. Flow through these denitrification bioreactor systems may be either horizontal or vertical. In laboratory studies, vertical flow systems, wherein influent water is pumped from the base of a column, tend to be preferred, as anaerobic conditions are easy to maintain and constant flow rates can be maintained.

3.2.1 Vertical flow systems

Different types of filter media have been examined in PRBs. Gilbert et al. (2008) studied seven types of materials (softwood, hardwood, coniferous, mulch, willow, compost and leaves) to select a suitable natural organic substrate to use in a PBR. Subsequent to a batch test, the material used in the laboratory-scale study was softwood. The columns were 0.09 m in diameter and 0.9 m long, and received an influent concentration of 50 mg NO_3-N dm^{-3} loaded from the column base at two HLRs: 0.3 cm^3 min^{-1} and 1.1 cm^3 min^{-1}. At the lower HLR, removals of more than 96% were measured, whereas removals of 66% were measured for the higher HLR. The impact of residence times was also studied by Claus and Kutzner (1985), who studied N removal in an up-flow packed bed reactor, with lava stones as support for the microbial growth. Using nitrate solution of different concentrations (1.8; 3.0; 4.3; 6.1 g NO^3 L^{-1}) and 5 different residence times (5; 3.3; 2.5; 2.0; 1.7 h), 95% denitrification was measured at the longest residence time.

Other types of filter media, such as shredded newspaper, have also been examined. Volokita et al. (1996) treated water in 0.55 m-high x 0.1 m-diameter laboratory columns using shredded newspaper (0.4 cm width). Complete nitrate removal of the inlet solution (100 mg L^{-1}) was achieved at an ambient temperature of 32°C. Sawdust has high denitrification rates due to its large surface area, but it is prone to clogging. Bedessem et al. (2005) used a mixture of sawdust and native soil in a 4.6 m-long, 7.6 cm-diameter laboratory column to treat synthetic wastewater. The total nitrogen (TN) removal was 31% in the control column (comprising only native soil) and 67% in columns with an organic layer (soil and sawdust). Saliling et al. (2007) evaluated woodchips and wheat straw using an up-flow bioreactor. The influent concentration was 200 mg NO_3-N L^{-1} and a 99% removal was obtained. Vrtovšek and Roš (2006) examined the effectiveness of a 1 m long x 0.12 m diameter fixed-bed biofilm reactor, comprising a mixture of PVC plastic and powdered activated carbon (PAC) as packing material. The reactor was inoculated with municipal wastewater before operation. Influent water with a concentration of 45 mg NO_3-N L^{-1} and sodium acetate ($CH_3COONa.3H_2O$) was loaded from the base of the column. Different loading rates were applied to the column, with drinking water quality being achieved at nitrogen loading rates (NLR) of lower than 1.9 g NO_3-N m^{-2} d^{-1}. Phillips and Love (2002) investigated a denitrifying bio-filter to remove nitrate from re-circulating aquaculture system waters using an up-flow fixed film column and two fermentation columns. Two nitrate concentrations (1.13 kg NO_3-N m^{-3} d^{-1} and 2.52 kg NO_3-N m^{-3} d^{-1}) were loaded at a HLR of 3.0 m hr^{-1}. The column was packed with polystyrene media with a specific surface area of 1000 m^2 m^{-3} and was seeded with activated sludge prior to operation. Commercial fish food was used as a fermentation source. Nitrate removal of greater than 99% was achieved. Rocca et al. (2007) used a coupling heterotrophic-autotrophic denitrification processes (HAD) supported by cotton and zero-valent iron (ZVI) to measure nitrate reduction. Two sets of columns filled with cotton and 150 g or 300 g of ZVI were used in this experiment. This had an up-flow inlet concentration of 100 and 220 mg NO_3 L^{-1}, and 3 and 6 mg L^{-1} of phosphate. The HAD had a higher volumetric nitrate removal ratio (VNR) than cotton-supported denitrification alone.

A laboratory sulphur-based reactive barrier system was evaluated by Moon et al. (2008) and was able to transform 60 mg N L^{-1} in di-nitrogen (N_2) in the presence of phosphate. The denitrification rate was higher than 95%. Cameron and Schipper (2010) compared nitrate removal, hydraulic and nutrient leaching characteristics of nine different carbon substrates. Mean nitrate removal rates for the period 10–23 months were 19.8 and 15 g N m^{-3} d^{-1} (maize cobs), 7.8 and 10.5 g N $m^{-3}d^{-1}$ (green waste), 5.8 and 7.8 g N m^{-3} d^{-1} (wheat straw), 3.0 and 4.9 g N m^{-3} d^{-1} (softwood), and 3.3 and 4.4 g N m^{-3} d^{-1} (hardwood) for the 14 and 23.5 C treatments, respectively.

3.2.2 Horizontal flow systems

Horizontal flow systems have also been used in studies. Healy et al. (2006) examined the use of various wood materials as a carbon source in laboratory horizontal flow filters to denitrify nitrate from a synthetic wastewater. The filter materials were: sawdust (*Pinus radiata*), sawdust and soil, sawdust and sand, and medium-chip woodchips and sand. Two influent NO_3-N concentrations, 200 mg L^{-1} and 60 mg L^{-1}, loaded at 2.9 to 19.4 mg NO_3-N kg^{-1} mixture d^{-1}, were used. The horizontal flow filter with a woodchip/sand mixture, loaded at 2.9 mg NO_3-N kg^{-1} d^{-1}, performed best, yielding a 97% reduction in NO_3-N at steady-state conditions. Using a sand tank containing a denitrifying zone in the centre (sand coated with soybean oil), Hunter (2001) measured a 39% nitrate removal of the initial concentration of 20 mg NO_3-N L^{-1} at a flow rate of 1112 L $week^{-1}$.

3.3 Wetlands

Dairy dirty water (DDW), defined in Section 2.3, can have a significant adverse effect on the environment. In Ireland, management of DDW is explained in Section 3.3, but in recent years, the use of constructed wetlands (CWs) for the treatment of DDW, as well as domestic and municipal wastewaters, has being gaining in popularity. This is due to their relatively low capital costs and maintenance requirements.

3.3.1 Wetland types

There are two types of CW: free water surface constructed wetlands (FWS CWs) and subsurface CWs. In FWS CWs, wastewater flows in a shallow water layer over a soil substrate. Subsurface CWs may be either subsurface horizontal flow CWs (SSHF CWs) or subsurface vertical flow CWs (SSVF CWs). In SSHF CWs, wastewater flows horizontally through the substrate. In SSVF CWs, wastewater is dosed intermittently onto the surface of sand and gravel filters and gradually drains through the filter media before collecting in a drain at the base. CWs may be planted with a mixture of submerged, emergent and, in the case of FWS CWs, floating vegetation. However, the ability of vegetation to capture nutrients, particularly in a cool temperate climate, is limited (Healy et al., 2007).

The large surface area of CWs provides an environment for the physical/physico-chemical retention and biological reduction of organic matter and nutrients (Knight et al., 2000; Lu et al., 2009). Depending on the type of CW used, its design, organic loading rate (OLR) and hydraulic retention time (HRT) (Karpiscak et al., 1999); a CW can have a significant nutrient removal capability. However, due to the effect of changing temperatures, the treatment efficiency of these systems tends to vary throughout the year (Bachand and Horne, 2000).

3.3.2 Design guidelines for dairy dirty water treatment

American guidelines for the design loading of SSHF CWs treating agricultural wastewater (NRCS, 1991) recommend an areal OLR of 7.3 g 5-day biochemical oxygen demand (BOD_5) $m^{-2}d^{-1}$; similar rates are used in wetland design for cool temperate climates (Cooper et al., 1996; Dunne et al., 2005ab). New Zealand guidelines for the disposal of DDW (Tanner and Kloosterman, 1997) recommended that an FWS CW should only succeed two waste stabilization ponds (an anaerobic and an aerobic pond, respectively) before entering the wetland with an OLR not exceeding 3 g BOD_5 m^{-2} d^{-1}. Generally, FWS CWs are used for the treatment of DDW as issues such as blockage of the filter media – normally associated with the operation of SSHF CWs – do not arise.

3.3.3 Treatment efficacy

Results from CWs have been variable. Table 4 tabulates the performance of FWS CWs in the treatment of DDW in a range of countries. In a study of planted and unplanted SSHF CWs, where the unplanted SSHF CWs acted as an experimental control, Tanner (1995ab) found that under 5-day carbonaceous biochemical oxygen demand ($CBOD_5$) OLRs ranging from 0.9 to 3.4 g $CBOD_5$ m^{-2} d^{-1} (unplanted) and 0.9 to 4.1 g $CBOD_5$ m^{-2} d^{-1} (planted), maximum $CBOD_5$ removals of 85% and 92%, respectively, were measured. Ammonification was more pronounced with increasing HRT, and total nitrogen (Tot-N) removal varied between 48 and 80% for planted CWs. Similar OLRs were used in a study on a 3-cell integrated FWS CW in Co. Wexford, Ireland (Dunne et al., 2005ab) where, under OLRs varying from 2.7 to 3.5 g BOD_5 m^{-2} d^{-1}, good organic removal was measured, but nitrification was not complete during winter.

Cronk et al. (1994) also found that under reduced retention times (with OLRs of 60 g BOD_5 m^{-2} d^{-1}) BOD_5 and suspended solids (SS) concentrations were not reduced to acceptable levels after treatment in a 1-cell FWS CW, and that no significant reduction of total kjeldahl nitrogen (TKN) occurred. In a study on a dairy farm in Drointon in the U.K (Cooper et al., 1996), a SSHF CW was used to treat influent with an average BOD_5 concentration of 1192 mg L^{-1}. The system initially utilized only the wetland alone and performed poorly under an OLR of approximately 26 g BOD_5 m^{-2} d^{-1}. However, when two SSVF CWs and a lagoon were

Parameter	Wetland Type	Loading rate	Influent ± SD	Effluent ± SD	Removal efficiency	Reference
BOD						
Ireland	FWS		998±1034	16±5	98	[1]
USA	FWS	~60	7130	2730	62	[2]
	FWS	~12	242	246	-2	[3]
	FWS	NP	1914	59	97	[4]
	FWS	18	2680	611	77	[5]
Australia	FWS	5.6	220	90	59	[6]
Italy	FWS	~1.9	451	28	94	[7]
N. Zealand	FWS	~4.1	113	27	76	[8]
	FWS	~1	337	11	92	[8]
COD						
Ireland	FWS		1718±2008	162±83	91	[1]
SS						
Ireland	FWS		535±434	34±31	94	[1]
USA	FWS	NP	5540	990	82	[2]
	FWS	NP	911	641	30	[3]
	FWS	NP	1645	65	96	[4]
	FWS	9	1284	130	90	[5]
N. Zealand	FWS	~8.5	150	33	78	[8]
	FWS	~1.9	142	34	76	[8]
Tot-N						
USA	FWS	0.7	103	74	28	[5]
	FWS	NP	170	13	92	[4]
N. Zealand	FWS	2.7	~38	20	48	[9]
	FWS	0.6	~38	10	75	[9]
NH_4-N						
Ireland	FWS		48±25	6±5	88	[1]
USA	FWS	0.05	8	52	0	[5]
	FWS	NP	72	32	56	[4]
Israel	FWS	NP	51	44	14	[10]
N. Zealand	FWS	NP	33	22	34	[9]
	FWS	NP	38	11	71	[9]
NO_3-N						
USA	FWS	NP	5.5	10	0	[4]
	FWS	$2x10^{-3}$	0.3	0.1	67	[5]
Tot-P						
USA	FWS	NP	53	2.2	96	[4]
	FWS	0.2	26	14	46	[5]
N. Zealand	FWS	0.8	~11	6.9	37	[9]
	FWS	0.2	~11	2.9	74	[9]
PO_4-P						
Ireland	FWS		15±7	3±2	80	[1]

Avg±SD; FWS = free-water surface constructed wetland; NP = not published
[1] Healy and O' Flynn (pers. comm.); [2] Cronk et al., 1994; [3] Karpiscak et al., 1999; [4] Schaafsma et al., 2000; [5] Newman et al., 2000; [6] Geary and Moore, 1999; [7] Mantovi et al., 2003; [8] Tanner et al., 1995(a); [9] Tanner et al., 1995 (b); [10] Ran et al., 2004.

Table 4. Average influent and effluent concentration (mg L^{-1}), loading rates (g m^{-2} d^{-1}), and removal efficiencies of wetlands treating dairy dirty water (DDW).

installed in front of the SSHF CW, the system had an OLR of approximately 4 g BOD_5 m^{-2} d^{-1} and had good organic and SS removal rates, but had limited nitrification due to large fluctuations in the inlet wastewater strength. Even under significantly reduced OLRs, SSHF and FWS CWs have under-performed. In Italy, a study on a 2 cell FWS CW operated in series and monitored over a 26 month period, treating a mixture of domestic and DDW at an average influent OLR under 2 g BOD_5 m^{-2} d^{-1}, showed that anoxic zones which developed in the wetland inlet meant that nitrification was inhibited, producing an effluent Tot-N which was mainly composed of ammonium-N (NH_4-N) (Mantovi et al., 2003).

Present agricultural practice in Ireland is governed by The European Communities (Good Agricultural Practice for Protection of Waters) Regulations 2009 (S.I. No. 101 of 2009), which places a responsibility on the individual farmer and the public authority to adhere to the conditions set out within the Nitrates Directive (EEC, 1991(a)) and other water quality directives to ensure good wastewater management practices. On account of this, CWs are becoming popular for the treatment of DDW. Healy and O′ Flynn (*pers. comm.*) evaluated the performance of seven CWs treating DDW in Ireland. They found that average removals of chemical oxygen demand (COD) from DDW were 91%. However, average effluent concentrations were 162 mg L^{-1}, which was much higher than the maximum allowable concentration (MAC). The performance of the CWs in the reduction of NH_4-N and ortho-phosphorus (PO_4-P) was also highly variable.

4. Conclusion

Much research focuses on the nutrient content of agricultural wastewaters and their inorganic fertilizer replacement potential. Many options for dairy slurry and dirty water are in place including land application, irrigation and treatment using a variety of on farm or off farm options. Nutrient, gaseous and microbial losses can result from land application of agricultural wastes. Much research focuses on matching crop requirements with organic fertilizer applications. In addition, the control of P within such wastes can prevent incidental losses to the environment e.g. chemical amendments. Once nutrients are lost, other forms of remediation such as PRB′s or wetlands may be applicable to protect a waterbody.

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