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Soil Contamination

Edited by Simone Pascucci

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Contributors

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Meet the editor

Dr. Simone Pascucci was born on May 10, 1976, in Rome, Italy. Master of science-level (2011) at the University of La Tuscia (Italy) in "Nature conservation in the marine environment". Laurea in Environmental Sciences (2008) at the University of La Tuscia. He has been working for the Italian CNR from 2002; he has taught lessons on the hyperspectral remote sensing data analysis and classification algorithms development, he was involved in many international field and airborne campaigns for target and anomaly detection and data fusion and correction; he wrote many technical reports, national and international papers. His current research activity is focused on application of hyperspectral imaging spectrometry (VNIR/SWIR/TIR spectral ranges) for (a) precision agriculture; (b) coastal waters quality assessment and monitoring; (c) urban materials, infrastructure condition and environmental pollutants characterization and its development, implementation and validation. Current position: technician at the CNR - Institute of methodologies for environmental analysis (Italy).

Contents

Preface

Soil contamination has severely increased over the last years, especially due to petroleum hydrocarbons, heavy metals and pesticides from industrial wastes and human activities. Even though in general soil quality research is facing an important technological challenge and several actions have been taken in order to assess, remediate and reduce the effects of contaminants on soils, suitable and standardized monitoring and remediation strategies of soil are required. In this sense, in the last decade there has been a growing emphasis on the utilization of residues and waste materials, coming from different industrial activities, in several remediation technologies (e.g., chemical degradation, photo-degradation) and bioremediation in order to clean up contaminated soils. The critical point regarding contaminated soil monitoring is the intrinsic difficulty in defining fixed monitoring variables and indicators as the establishment of any a priori criterion and threshold for soil quality can be still considered subjective.

The book aims at collecting contributions from outstanding scientists and experts involved in different fields of soil contamination in order to show new research highlights and future developments in the context of contaminated soil monitoring and remediation strategies. The book is organized into eight auto-consistent chapters regarding application-oriented studies in the field of soil contamination.

The chapters include selected topics covering long-term monitoring studies of dioxin and furan level in soils; contamination of factory and roadside soils by hydrocarbons and heavy metals; soil contamination caused by winter maintenance in cold regions; the use of reflectance spectroscopy and hyperspectral remote sensing for soil contaminants and waste material detection; an updated review of the use of bioindicators and biomarkers for the assessment of soil toxicity and of soil transmitted pathogens in humans and associated risk factors; and also a consistent review of different remediation technologies and strategies (bio-phytoremediation) of contaminated soils.

I hope that the collected materials will provide to soil contamination researchers, experts (e.g., geologists, engineers and biologists), practitioners at universities, and other interested end-users a scientific basis and practical guide in the field of soil contamination to widen their experience to the presented topic areas.

All issues regarding soil contamination included in the book are significant and I want to thanks the authors for their precious contribution.

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1

Long-Term Monitoring of Dioxin and Furan Level in Soil Around Medical Waste Incinerator

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1. Introduction

The annual generation of solid waste is quite huge in China. For instance, approx. 157 million tons of municipal solid waste (MSW) and 2.04 billion tons of industrial solid waste (14.29 million tons of hazardous waste) were produced in 2009 (National Bureau of Statistics of China, 2010). These wastes would contaminate green land, drinking water and even air, ultimately threatening human health, so they must be treated in scientific methods. Waste treatment is a big challenge for every country. At present, the conventional disposal system according the hierarchy of methodologies includes recycle, compost, combustion and landfill. Combustion has noticeable advantages in volume and weight reduction, disinfection and short time cost, can also realize energy recovery by using waste to energy plants. Thermal treatment (pyrolysis and incineration) is the widely applied technology for waste treatment, for instance, accounting for 18.2% of MSW treatment in China and 11.9% in USA (2009). There are over 300 central incinerators for hazardous solid waste (HSW) in China (National Development and Reform Commission of China, 2003) and 93 municipal solid waste incinerators (National Bureau of Statistics of China, 2010). The present Chinese regulations prohibit the co-combustion of HSW and MSW (Ministry of Environment Protection, 2001).

However, waste incineration is still a controversial issue among social and scientific communities due to its secondary pollution, especially after the observation of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in incinerators (Olie et al., 1977). Waste incineration is thought a major source of PCDD/Fs in the environment. UNEP (UNEP Chemical, 2005) published the standardized toolkit for identification and quantification of dioxin and furan, including the emission factor of PCDD/Fs from combustion and incineration. Research (Gao et al., 2009; Ni et al., 2009) shows the emission factor of PCDD/Fs from medical waste incinerators (MWI) is nearly 63.3 μg I-TEQ/ton refuse into the atmosphere and 1.73 μg I-TEQ/ton from municipal solid waste incinerators (MSWI) in China, respectively. There are 135 dioxins and 175 furans, each with a different number and position of the chlorine atoms. 17 congeners of PCDD/Fs with 2,3,7,8 positions substituted by chorine are very toxic, which can induce a variety of adverse health problems, such as sarcomas, lymphomas and stomach cancer (Mitrou et al., 2001). These toxic pollutants can be formed by de novo synthesis and from precursor compounds (McKay, 2002), be emitted into the air through the stack, and transported to the ambient air, then deposited over a wide area of earth surface (Wu et al., 2009). It's essential to control pollutant emission to minimize the environmental and health impact. A lot of relevant researches on dioxin determination, formation and emission control have been conducted in last decades. Unfortunately, all of this work still can not completely eliminate the public concern. Incinerators construction and operation is opposed by public and environmental protection organizations for PCDD/Fs exposure risk. Public protests happened a couple of times in last two years, and the constructions of several plants were halted in China.

In order to clarify dioxin exposure risk, surveys and monitoring programs have been carried out via detecting PCDD/Fs concentration in environmental media including soil, water, air, food and bio-tissues. On one hand, there are remarkable influences of waste incinerators on the environment. Kim et al. (2005) measured PCDD/Fs concentrations in ambient air, soil, pine needles and human blood in order to assess the relationships between incinerator sources and environment. It was observed the incinerator operation had directly influenced the observed PCDD/F congener profiles of soil and pine needles. Further, the difference between the levels of PCDD/Fs in the blood of office and plant workers demonstrates that human exposure to PCDD/Fs occurs as a result of the operation of the incinerator. By the Korea national monitoring of PCDD/Fs in the environmental media around incinerators (Kim et al., 2008), the average PCDD/Fs levels in soils decreased with increasing distance from the incinerator. From the PCDD/Fs level gradient away plant, a distance of 500 m is suggested as being under the influence of an incinerator. After introduction of technical improvement in MSWI, a reduction of 40% was observed in the median PCDD/Fs level in soil around the facility (Domingo et al., 2002). On the other hand, no significant impact of a waste incinerator on the neighborhood was reported too. In the research of a 10-year surveillance program of a hazardous waste incinerator (HWI) (Vilavert et al., 2011), the median value of PCDD/Fs in soil samples decreased 44% (from 0.75 to 0.42 ng I-TEQ Kg^{-1}) between 1999 and 2009 year survey. In order to establish the temporal variation after 6 years regular operation, the concentrations of PCDD/Fs in blood and urine of 19 workers employed at a HWI were measured in 1999 and 2005 (Mari et al., 2007). The analyzed results indicate that the workers at the HWI are not occupationally exposed to PCDD/Fs in their workplaces. In our previous research (Xu et al., 2009), the overall PCDD/F levels in the soil collected from the vicinity of the MSWI increased significantly, i.e., 39% for I-TEQ (median value) between 2006 and 2007, though the impact of MSWI on this study area is limited by congener-specific factor analysis. By the above review of the environmental impact of incinerators, this topic is still not resolved. The main potential reason is the different operation condition and pollutant emission level.

PCDD/Fs emission factor of MWI is much higher than the value of MSWI (UNEP Chemicals, 2005), so it is presumed that MWI has worse environmental influence than MSWI. In this study, PCDD/Fs level in soil in the vicinity of a MWI was monitored since April 2007, before this plant started operation (May 2007), and continued this determination every year after operation (2008-2010). This studied MWI is a typical central incinerator in China, with a capacity of 20 ton/day. The detailed sampling/analysis methods and experimental results are introduced along with discussion in this chapter.

2. Method and material

2.1 Study region and MWI

This studied MWI locates in the north of Zhejiang province, China. The designed capacity is 20 tons waste per day. The combustion technology is a rotary kiln combined with a secondary combustor, as well as, an off-gas cleaning system that contains a quencher, a semi-dry scrubber and a fabric filter. There is another pyrolysis furnace (5 tons/day) in this factory, and its exhaust gas is emitted through the same stack as the incinerator. So the stack position is defined as this MWI location. The height of this stack is 35 m, and it is still lower than the near hills (Fig.1).

Fig. 1. Outside view and internal view of the medical waste incinerators.

2.2 Soil sampling method

Twelve soil samples for each year were collected in the vicinity of the MWI as shown in Fig.2. The exact sampling points were determined and recorded within 10 m of accuracy by a handheld GPS device (Meridian Color, Thales Navigation, USA), then transformed each point into the Geographic Information System (GIS) software packages of Google Earth (2003).

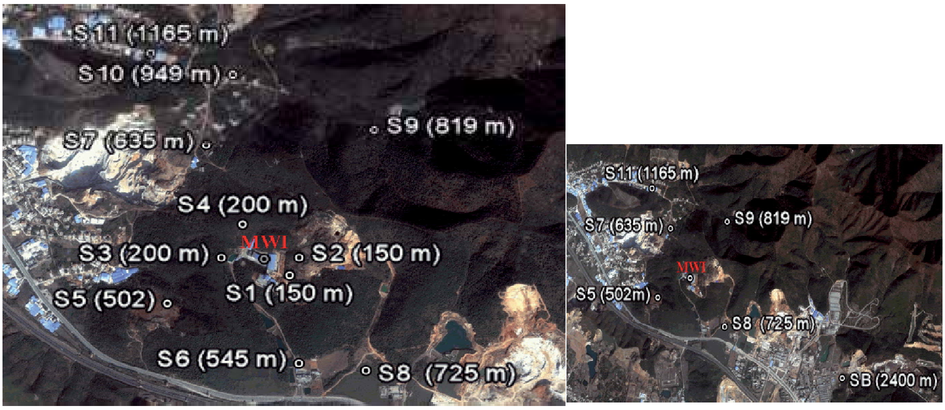

Fig. 2. Soil sample sites around the studied MWI.

The background sample (SB) was collected in a farmland southeast of the stack, 2400 m away. The local climate is featuring distinct seasons, typical to a subtropical weather condition. The seasonal wind is from the southeast direction in summer and northwest in winter. The sampling sites are mainly distributed in southeast and northwest. The MWI is built in a valley area, so that the choice of sampling sites must consider the site-condition. As some sites were frequently cultivated by farmer, the sampling was carried out by inserting a cylindrical steel corer (24cm × 4cm, length × internal diameter, Eijkelkamp, Holland) down to a 10 cm depth. To obtain composite samples for each sampling point, soils were collected by mixing five different components (four main directions of 2 m radius and the center) within a 12.6 m^2 area. Approx. 1.5 kg of soil was taken at each site. Soil samples were air-dried in a ventilated room until reaching constant weight, and bio-material (roots, leaves) was manually removed. Then they were skived and sieved to < 0.25 mm. They were refrigerated until analysis, within two weeks. The first survey as PCDD/Fs baseline was conducted at April 2007, before this MWI started operation (May 2007). And soil samples were collected every year (2008 to 2010) in the same sites as the first survey after this facility operation began. During this period, fly ash and stack gas samples were collected from this MWI.

2.3 Clean procedure and analysis technology

About 10 g (dry mass) of soil samples were used for PCDD/Fs analysis. A selective pressured liquid extraction (SPLE) method was used for sample extraction by using a fully automated ASE 300 system (Dionex, Sunnyvale, CA, USA) (Fig.3). The extraction condition and procedure was referred to the SPLE method with a slight modification. Briefly, a 100-ml extraction cell was used and the ratio of soil:alumina:copper was 5:5:1. Each sample was spiked with a mixture of $^{13}C_{12}$-labelled PCDD/Fs compound stock solution (5 μl) and clean-up standard (5 μl) before extraction. The extracts from ASE were subsequently followed by rotary evaporation and multilayer silica gel column clean-up procedure following the Method of USEPA 1613. The extracts were blow-down to 20 μl under a gentle stream of nitrogen (N_2), and 5μl of $^{13}C_{12}$-labelled PCDD/Fs internal standard solution were added before sample were subjected to PCDD/Fs analysis by using high-resolution gas chromatography with high-resolution mass spectrometry (HRGC/HRMS) (JEOL JMS-800D) with a DB-5MS column (60 m × 0.25 mm × 0.25 μm). The toxic 2,3,7,8-substituted PCDD/Fs (referred to as congeners) as well as Tetra- to Octa-chlorinated homologues were identified based on isotope, and quantification of PCDD/Fs was performed by an isotope dilution method using relative response factors previously obtained from the five calibration standard solutions. In order to check the duplicate results, two soil samples are analyzed twice each year survey. If there is a wide variation in samples results, it also will be analyzed again. All isotope standards were purchased from the Cambridge Isotope Laboratories, Inc. (USA).

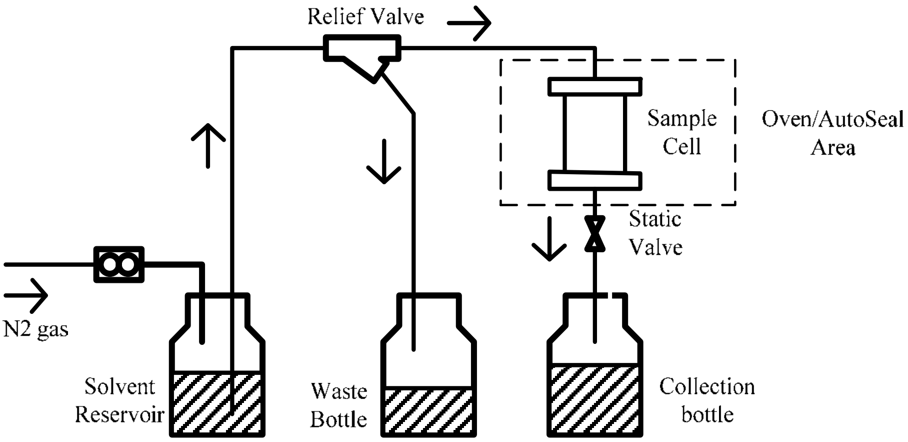

Fig. 3. ASE 300 Schematic System.

For source identification by comparison of PCDD/Fs homologue/congener patterns between soil and MWI emissions, stack gas and fly ash were collected from this MWI. The stack gas samples were collected with an isostack sampler (M5, KNJ Engineering, Korea) according to USEPA method 23A. The sample collection components included a glass fiber filters, in line with a condenser, the sorbent (XAD-2 resin) module and four impingers. The sampling labelled-$^{13}C_{12}$ standard was spiked into the XAD-2 resin before the sampling of flue gas. And the clean procedure was conducted as EPA23 method, including Soxhlet extraction by toluene for 24 h, wash with sulfuric acid (H_2SO4), a multi-layer silica gel column and an alumina column. The final clean extracts were blow-down to 20 μl under a gentle stream of nitrogen (N_2).The fly ash was collected at the exit of the bag filter. The clean procedure was conducted as EPA1613. The difference between EPA23 and EPA1613 is just using different labeled-$^{13}C_{12}$ standard solution as EPA1613 without sampling standard solution, and the clean process is generally the same. All of these samples were analyzed by HRGC/HRMS. The more detailed procedure of clean-up flue gas and fly ash samples can be found in the previous report (Chen et al., 2008).

2.4 Data analysis

All the experimental results were expressed on a dry weight basis. The 2,3,7,8-TeCDD toxic equivalents (I-TEQ) were calculated using NATO/CCMS factors (1988). Data was normalized before comparison of homologue and the multivariate analysis. Principal component analysis (PCA) was used to evaluate the similarities and differences of the PCDD/Fs homologue patterns and HxCDF isomer profile in soil samples, flue gas and fly ash. Each sample was assigned a score after PCA, allowing the summarized data to be further plotted and analyzed. PCA was performed using the SPSS 16.0 software package.

3. Results and analysis

The analysis results are present in table 1, including amount and TEQ concentration. Amount refers the concentration of total PCDD/Fs homologue from Tetra- to Octa-chlorinated species. PCDD/Fs level displays significant variation during these four years.

Sites	Amount, pg·g^{-1}				TEQ, pg I-TEQ·g^{-1}			
	2007	2008	2009	2010	2007	2008	2009	2010
S1	58.26	439.84	258.96	290.41	0.78	2.21	3.17	4.74
S2	848.34	1981.89	1155.45	1279.39	2.63	5.78	3.54	5.11
S3	397.04	465.10	374.05	669.21	1.78	3.51	2.37	6.07
S4	78.44	626.59	170.45	293.11	0.97	4.83	2.55	4.35
S5	433.77	546.01	551.95	1012.10	1.04	1.04	1.84	3.34
S6	66.48	89.55	123.51	164.73	0.64	0.94	1.34	1.41
S7	44.34	175.91	66.82	97.59	0.46	1.77	0.85	0.98
S8	263.18	273.81	252.84	329.64	1.91	1.99	1.47	3.30
S9	81.64	133.31	125.84	159.62	1.08	1.25	0.91	1.07
S10	57.18	78.51	67.49	92.80	0.45	0.88	0.69	1.12
S11	76.71	163.60	106.04	269.31	0.71	0.98	1.01	1.87
SB	55.94	55.72	79.42	85.01	0.60	0.53	0.73	0.65
Mean	205.11	419.15	277.73	395.24	1.09	2.14	1.70	2.83
Median	77.57	224.86	148.14	279.86	0.87	1.51	1.40	2.59

Table 1. PCDD/Fs amount and I-TEQ concentration in soil samples.

3.1 Baseline of PCDD/Fs concentration in soils

In the baseline survey (2007), PCDD/Fs concentration in this studied region is in the range of 44.34 to 848.34 pg g^{-1} (0.45 - 2.63 pg I-TEQ g^{-1}) with a mean of 205.11 pg g^{-1} (1.09 pg I-TEQ g^{-1}). It is lower than 4.0 pg I-TEQ g^{-1}, which is PCDD/Fs limit standard for cultivation land soil (GB15618-2009) in China (Ministry of Environment Protection, 2009), and this reflects there is no remarkable PCDD/Fs contamination. The German guideline (Federal Ministry for the Environment, 1992) recommends a limit of 5 pg I-TEQ g^{-1} for unrestricted agricultural use. US EPA (1998) recommends 1 pg I-TEQ g^{-1} in residential soil and 5 pg I-TEQ g^{-1} in commercial soil. Zheng et al. (2008) did a review of PCDD/Fs source and level in China, and found 0.09 to 2.4 pg I-TEQ g^{-1} in mountain and 0.14 to 3.7 pg I-TEQ g^{-1} in farmland. According to the survey (Jou et al., 2007), it is observed that PCDD/Fs range from 0.10 to 8.48 pg I-TEQ g^{-1} with an average of 2.20 pg I-TEQ g^{-1} in soil collected from a nature preserve area in Taiwan. Dioxin level in a urban surface soil in Norway is in the range of 0.16 to 14 pg I-TEQ g^{-1}(Andersson & Ottesen, 2008), and PCDD/Fs baseline in rural soil in Spain is 0.17 – 8.14 pg I-TEQ g^{-1} (Schuhmacher et al., 2002). Therefore, PCDD/Fs level in this survey is lower or generally comparative with the value of other places, beyond remarkable pollution. Further, the highest concentration is in S2, which is obviously abnormal from other sites. Actually, the surface and soil character in S2 is quite special, where is completely bare without any plant or herb, the soil is like limestone, which is commonly used in construction. So it is presumed that this point was polluted by some unknown historic activity, especially during the MWI construction.

3.2 PCDD/Fs concentration and variation after MWI operation

After this MWI started operation, a significant variation of PCDD/Fs concentration in soil is observed. In 2008, PCDD/Fs concentration ranges from 55.72 to 1981.89 pg g^{-1} (0.53 – 5.78 pg I-TEQ g^{-1}) with an average value of 419.15 pg g^{-1} (2.14 pg I-TEQ g^{-1}). In 2009, PCDD/Fs level is 66.82 – 1155.45 pg g^{-1} (0.69 – 3.54 pg I-TEQ g^{-1}) with an average of 277.73 pg g^{-1} (1.70 pg I-TEQ g^{-1}). In 2010, PCDD/Fs level ranges from 85.01 to 1279.39 pg g^{-1} (0.65 – 6.07 pg I-TEQ g^{-1}) with an average of 395.24 pg g^{-1} (2.83 pg I-TEQ g^{-1}). In the 2010 survey, the extraordinary sample is S5, and the increase compared to the value in 2009 is up to 460.15 pg g^{-1} (1.50 pg I-TEQ g^{-1}). So it is re-analyzed, and there is almost no difference between two measurements. In the on-site place of S5, there is no obvious specific pollution source. S5 is located in a hillside without herb or plants, and rain wash up is noticeable there. The possible explanation is that pollutants on soil surface were washed by rain and enriched in S5. Certainly, the persistent pollutant concentration in soil is the multi-result of pollution, distribution, deposition and bio-degradation.

The overall variation of PCDD/Fs level in soil is shown in Fig.4 and Fig.5. Figure 4 is the box plot of PCDD/Fs concentration each year, and Fig.5 is the comparison of PCDD/Fs baseline and the average of PCDD/Fs level after MWI operation (2008 to 2010) in every sites. In Fig.4, the PCDD/Fs variation is clear. PCDD/Fs level after operation is always higher than the baseline, and there is a little drop in 2009 compared to 2008. As analyzed in the previous paper (Li et al., 2010), the dioxin emission from this factory was largely reduced because medical waste combustion decreased and a series of improvements according to best available technique and best environment practice (BAT/BEP) were implemented in August 2008 (Lu et al., 2008). After the improvement, PCDD/Fs concentration in the stack gas and fly ash reduced by 96.7% and 83.15 %, respectively. This is the major reason of the PCDD/Fs decrease in the 2009 survey. In Domingo's research

(2002), a similar result was observed around a MSWI, 40% reduction in soil after technical alteration in the MSWI. Lee et al. (2007) found PCDD/Fs concentration in air around MSWI decreased approx. 50% after the introduction of a new flue gas treatment, as well as, 99.98% reduction of PCDD/Fs in stack gas samples. However, the PCDD/Fs level continues to increase in 2010 survey. The PCDD/Fs distribution in different sites and the relation of PCDD/Fs variation with distance from MWI is present in Fig.5. In the baseline, all of the sites almost stay in the same level of PCDD/Fs, and there is no specific trend with distance. After operation, the level curve (AO) goes up, particularly in the close sites (S1 to S4). With the amount comparison, the largest increase of PCDD/Fs (629.31 pg g^{-1}) is in S2, which is the closest point from MWI. Furthermore, S1 is the same distance away the stack as S2, and its increase (271.47 pg g^{-1}) is much lower than S2′ increase. The main reason is the different characteristic surface in these two sites, as the thick grass covers in S1. Grass can reduce the adsorption of PCDD/Fs in soil, even absorb and degrade these toxic substances. And the curve (AO) of TEQ after operation displays a slight decline with distance. Meanwhile, the variation of PCDD/Fs is not significant in the farther sites than S5. So approx. 500 m radius is thought as the influence area in this case, which is consistent with another study (Kim et al., 2008). In this possible influenced area, there are no inhabitants except the staff of this plant, so the workers had better take strict protection to avoid health risk.

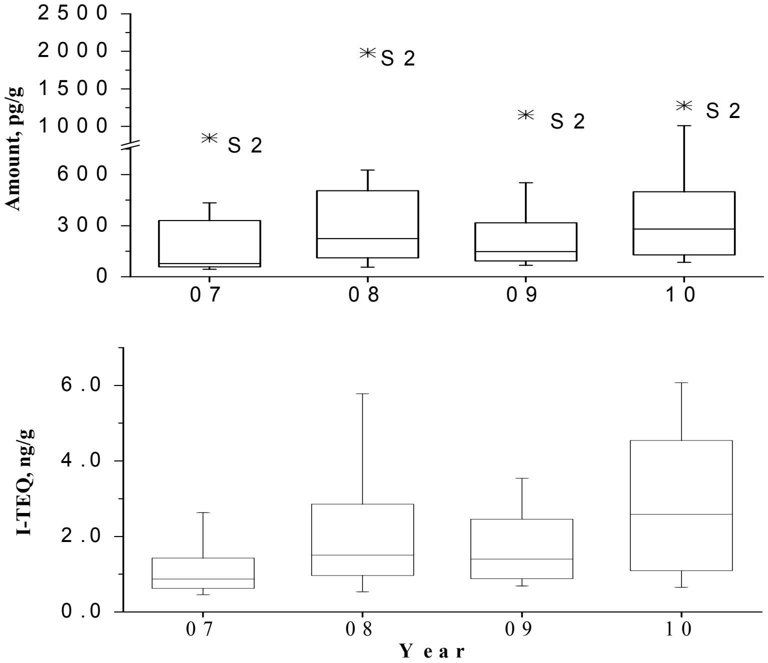

Fig. 4. Box plot of PCDD/Fs concentration in soils.

Figure 6 summarizes the average PCDD/Fs level in soil samples in the 2010 year survey and the comparison with different sites from Spain (Jiménez et al., 1996; Domingo et al., 2000), Taiwan (Cheng et al., 2003), Italy (Caserini et al., 2004; Capuano et al., 2005), Switzerland (Schmid et al., 2005), Norway (Andersson & Ottesen, 2008), South Korea (Kim et al., 2008), China (Yan et al., 2008), USA (Lorber et al., 1998) and Japan (Takei et al., 2000). The present PCDD/Fs level in this studied region is in the normal level as shown in Fig.6.

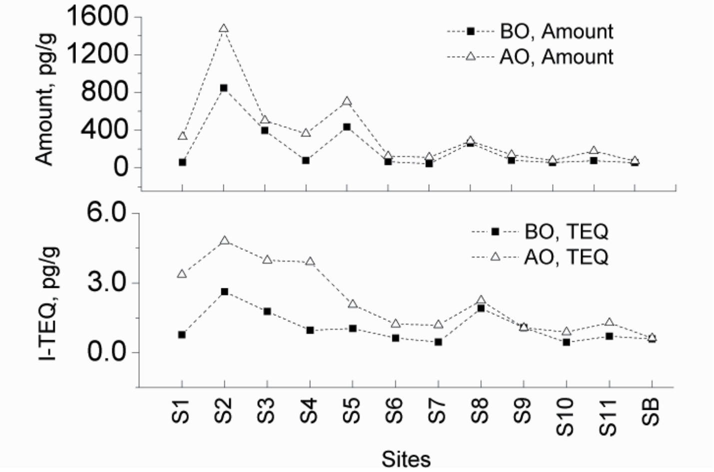

Fig. 5. Comparison of PCDD/Fs in soils collected before operation (BO, 2007) and after operation (AO, average of 2008 to 2010).

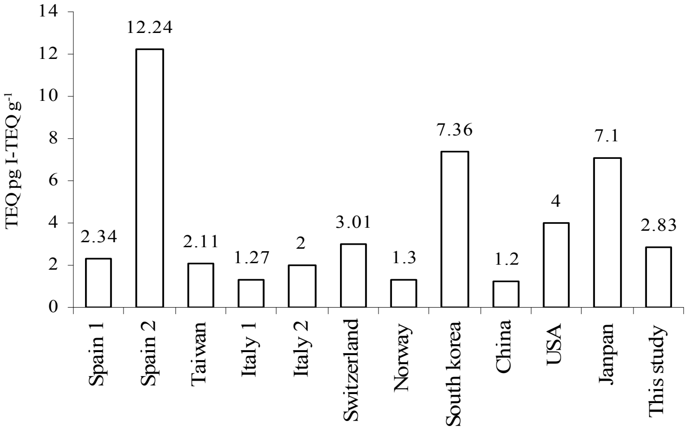

Fig. 6. The average of PCDD/Fs level in soil around worldwide.

3.3 Analysis of PCDD/Fs homologue pattern

Jiménez et al. (1996) found a slight PCDD/Fs contamination in soil near a medical waste incinerator in Madrid Spain, but did not clarify whether this plant was the only PCDD/Fs source responsible for the contamination. Homologue pattern or specific congener/isomer is defined as the fingerprint of PCDD/Fs. PCDD/Fs homologue distribution in soil, fly ash and stack gas are present in Table 2 to 6. The average PCDD/Fs homologue pattern in different surveys is present in Fig.7. Different PCDD/Fs sources have different fingerprint (Alcock et al., 1999; Domingo et al., 2001). In generally, the ratio of PCDFs to PCDDs from combustion processes is larger than 1, and a maximum weight distribution is PeCDF or HxCDF (Huang & Buekens, 1995). OCDD predominates PCDD/Fs homologue in the soil samples, which is consistent with other surveys. The deposition of OCDD on soil is easier and OCDD has longer degradation half-life time (Sinkkonen & Paasivirta, 2000). In the stack gas and fly ash, the dominant compound is HxCDF and PeCDF, and OCDD proportion is less than 5%. In 2007 survey, percentage of OCDD is in the range of 40.81 to 90.97 with an average of 58.51, and the average ratio of PCDFs to PCDDs is 0.40. In 2010, the average percentage of OCDD distribution is 43.51 and the mean ratio is 0.72. That means the proportion of OCDD decreases and the ratio of PCDFs to PCDDs increases, and this change might be caused by PCDD/Fs source from combustion or other thermal processes.

2007	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	SB
TeCDD	7.40	0.24	2.15	2.08	0.86	5.18	2.82	4.81	2.12	1.44	1.61	3.00
PeCDD	3.19	0.12	0.77	1.00	0.18	2.58	2.59	2.41	0.64	ND	2.49	3.61
HxCDD	2.02	0.39	1.23	3.42	0.72	3.82	3.80	2.15	2.46	2.51	1.23	3.86
HpCDD	7.49	2.14	5.07	7.60	3.55	7.07	7.74	5.44	4.93	5.25	7.41	6.38
OCDD	41.0	91.0	79.3	48.7	88.3	42.2	41.3	58.0	59.4	59.2	56.6	40.8
TeCDF	17.2	1.76	4.30	9.80	2.82	16.2	9.51	14.5	6.02	8.09	5.87	13.8
PeCDF	6.23	0.79	1.55	8.12	1.00	6.13	7.41	3.34	7.43	6.11	6.57	4.69
HxCDF	7.37	1.31	3.01	9.13	1.28	7.85	9.35	4.26	7.22	8.94	7.28	10.2
HpCDF	6.95	1.16	1.39	8.04	0.89	5.99	10.24	2.99	6.31	5.21	6.31	7.52
OCDF	1.15	1.13	1.24	2.10	0.44	2.98	5.26	2.17	3.46	3.21	4.68	6.14

Table 2. PCDD/Fs homologue distribution in soil of 2007, %.

2008	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	SB
TeCDD	0.80	0.26	1.76	0.83	0.20	2.31	1.97	2.59	1.92	3.10	1.26	2.02
PeCDD	0.74	0.29	0.78	0.85	0.31	2.94	1.60	2.64	2.31	2.45	1.51	2.24
HxCDD	1.14	0.27	2.63	2.09	0.67	4.58	2.55	2.81	3.43	4.44	1.69	6.78
HpCDD	1.17	1.55	5.43	3.49	3.44	5.16	3.08	4.60	4.17	5.59	3.22	4.42
OCDD	58.4	73.8	56.3	36.1	91.6	32.6	18.5	43.6	40.8	28.9	26.8	43.3
TeCDF	6.70	1.87	10.2	4.94	1.37	13.0	15.7	11.4	8.92	14.2	6.19	17.7
PeCDF	6.28	1.33	8.40	5.89	0.77	13.8	14.8	12.3	6.45	7.42	4.68	11.4
HxCDF	5.78	1.47	8.00	5.27	0.66	12.11	14.3	8.16	7.68	12.3	4.64	5.48
HpCDF	2.87	1.43	4.33	5.22	0.60	7.21	7.77	4.56	7.27	11.4	6.05	3.96
OCDF	16.1	17.7	2.22	35.4	0.35	6.33	19.8	7.38	17.1	10.3	44.0	2.72

Table 3. PCDD/Fs homologue distribution in soil of 2008, %.

2009	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	SB
TeCDD	3.06	0.36	1.14	3.40	0.68	3.09	3.10	3.49	1.77	2.82	3.05	2.57
PeCDD	3.41	0.45	1.42	3.21	0.68	5.00	4.42	3.37	1.86	3.62	3.40	2.70
HxCDD	4.51	0.63	2.37	5.81	1.04	4.30	5.17	2.92	3.68	3.74	4.02	3.90
HpCDD	4.42	2.02	5.24	5.23	3.69	5.17	5.67	5.10	4.35	6.16	5.63	4.66
OCDD	41.9	88.2	70.0	25.8	84.2	33.1	37.7	55.8	52.9	40.5	39.1	44.4
TeCDF	14.4	2.53	5.53	20.7	3.29	13.1	11.0	9.50	11.7	10.6	19.5	11.8
PeCDF	9.36	1.71	5.14	10.5	2.16	8.18	9.65	9.91	8.89	7.28	7.65	11.6
HxCDF	10.3	1.68	4.37	11.9	1.87	9.67	11.6	4.71	6.88	11.0	8.08	8.00
HpCDF	6.50	1.15	3.13	8.84	1.55	8.52	7.09	3.27	4.76	9.22	6.09	5.93
OCDF	2.23	1.23	1.71	4.52	0.83	9.86	4.57	1.98	3.16	5.15	3.46	4.40

Table 4. PCDD/Fs homologue distribution in soil of 2009, %.

2010	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	SB
TeCDD	5.85	0.53	1.79	3.61	0.46	2.21	4.17	2.26	1.65	3.05	1.72	2.79
PeCDD	5.53	0.66	2.06	5.73	0.51	4.33	4.96	3.06	2.61	6.24	2.71	1.75
HxCDD	7.45	1.15	3.73	6.40	0.94	4.89	4.30	4.00	3.64	5.71	2.19	6.02
HpCDD	4.60	2.52	5.30	5.05	3.19	6.16	5.47	5.28	4.45	5.41	2.92	5.14
OCDD	14.2	83.4	56.8	21.7	78.8	49.7	34.9	47.7	52.8	32.1	20.8	29.4
TeCDF	16.9	3.06	9.50	17.9	11.3	9.35	14.6	7.86	13.1	15.9	10.8	20.3
PeCDF	14.1	2.35	6.26	12.8	1.37	6.45	8.24	6.06	6.99	8.01	2.71	12.5
HxCDF	15.5	2.37	6.92	13.5	1.40	7.20	9.25	8.17	5.75	8.88	5.65	9.26
HpCDF	11.4	1.86	5.33	9.01	1.20	6.37	8.57	11.7	5.54	9.20	13.6	8.68
OCDF	4.49	2.16	2.26	4.25	0.82	3.38	5.62	3.84	3.44	5.58	36.9	4.21

Table 5. PCDD/Fs homologue distribution in soil of 2010, %.

	TeCDD	PeCDD	HxCDD	HpCDD	OCDD	TeCDF	PeCDF	HxCDF	HpCDF	OCDF
Fly ash	3.57	6.76	10.76	7.19	3.39	18.48	11.39	20.51	14.71	3.24
Stack gas	3.02	6.99	5.44	3.93	2.31	20.33	17.20	23.64	13.40	3.73

Table 6. PCDD/Fs homologue distribution of fly ash and stack gas, %.

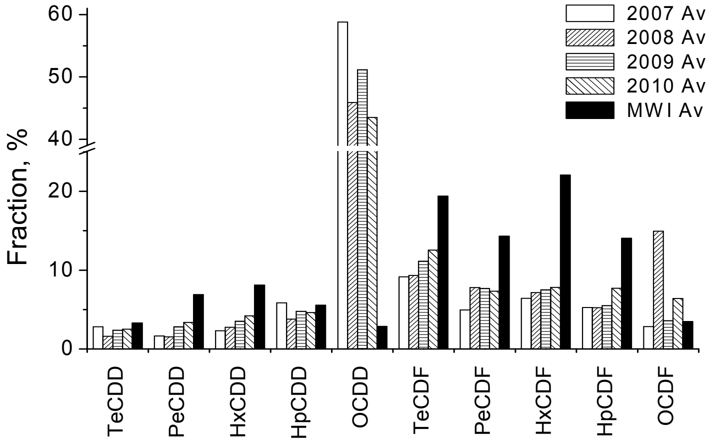

Fig. 7. PCDD/Fs Homologue pattern of soil and MWI samples (Av, Average).

Principal component analysis (PCA) is used to estimate the similarity and difference of homologue pattern between soil and the presumed source (MWI), as shown in Fig.8. Accumulation information of component 1 and component 2 is up to 77.98%, means these two components can well represent the total information of all samples. Component 1 mainly depends on OCDD, HxCDF and HxDD, as well as component 2 is related to OCDF and HpCDD. The sites of fly ash and stack gas locate on the right of the PCA score plot, separates from soil samples, which indicates a clear difference between MWI emission and soils in the homologue distribution. Overall, 2007 survey soils are mainly located top left, 2008 soils are mainly in bottom, 2009 and 2010 year soils are mainly in the centre. The groups of each year illuminate homologue patterns in soil change with time, and show a close relation in the soils collected 2009 and 2010. Considering the average distance between each year soil group and fly ash (stack gas), soils points move closer to fly ash and stack gas with the time, especially S1 and S4 of 2010 year. It demonstrates there is a possible influence of the MWI in neighboring soil that accumulates with year's past. By the way, the fly ash and stack gas samples can not completely display MWI characteristic emission because PCDD/Fs emissions change with different operation parameters. And other combustion process like open burning, firewood usage, and vehicle might release similar PCDD/Fs. In addition, since fly ash is a major output of PCDD/Fs in incinerators (over 50%) (UNEP Chemicals, 2005; Huang & Buekens, 1995), a good and scientific collection and storage of fly ash must be conducted, to avoid leaking and diffusing into the surrounding environment.

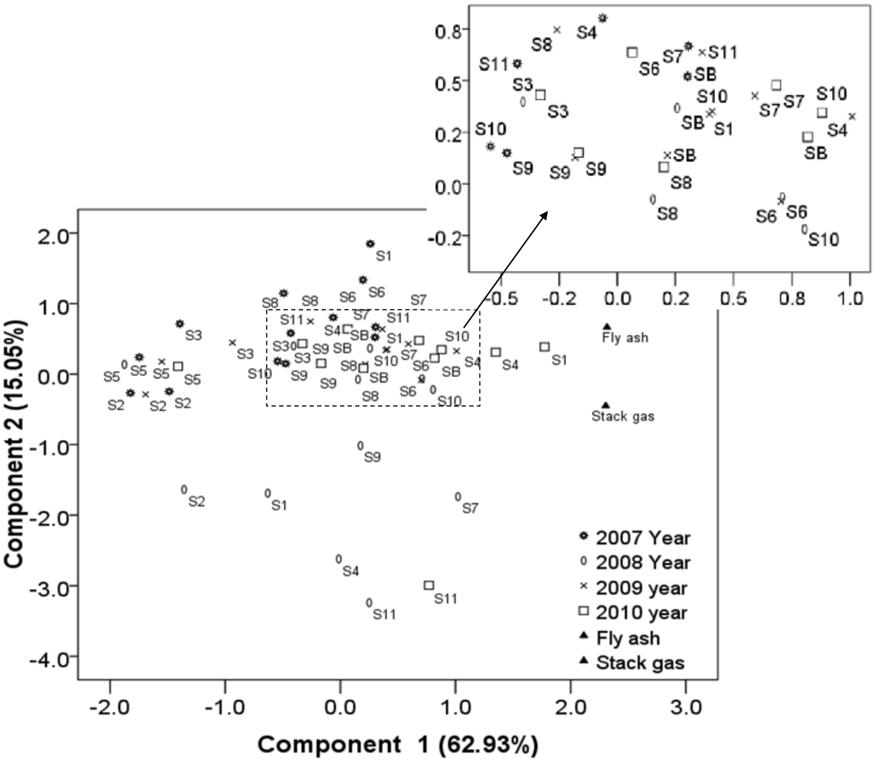

Fig. 8. PCA plot of PCDD/Fs homologue.

3.4 Analysis of HxCDFs isomer profile

PCDD/Fs from Tetra- to Octa-chlorination have ten homologues with different molecular structure and different substituted chlorines, and these compounds have different chemical and biological properties. PCDD/Fs are emitted from source, deposited on earth surface, distributed and decomposed in soil and organism, lot different activities would happen in this process, which deteriorate the relation of soil and source in PCDD/Fs homologue pattern. In order to minimize these possible changes, further analysis focuses on isomer profile of the same homologue. The isomer pattern is expressed as the relative percentage of an isomer with each homologue, which is useful for source identification to compensate for homologue-dependent difference (Ogura et al., 2001; Xu et al., 2008). HxCDF is the dominant homologue in MWI samples (Table 6), so HxCDF is chose to investigate the isomer profile. Table 7 to 10 are HxCDFs isomer distribution in soil samples, stack gas and fly ash, respectively. There are 16 isomers of HxCDF besides 4 toxic species whose 2,3,7,8 position are occupied by chlorine atom. 124678-HxCDF is the same peak with 134678-HXCDF in gas-chromatographic elution, 123679-HxCDF is also the same peak with 123469-HxCDF, so these two isomers are not assigned; meanwhile, 123489-HxCDF is difficultly separated from 123789-HCDF, so 123489-HxCDF is not assigned too. Fig.9 shows the average of HxCDF isomer pattern in different surveys, the dominated species is 134678-HxCDF, as well as, 123467-HxCDF, 123478-HxCDF and 123678-HxCDF. The average isomer profile among soil and MWI emission (Fig.9) is more similar than the average homologue pattern (Fig.7).

Position	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	SB
123468	6.06	7.43	7.97	7.75	9.64	11.71	9.60	10.7	5.95	9.14	11.08	8.02
134678	44.0	21.62	33.2	32.43	37.0	18.8	28.1	33.3	21.9	32.8	9.79	34.4
134679	ND	ND	1.98	3.91	1.23	0.90	ND	ND	ND	ND	7.75	ND
124679	7.88	2.16	ND	ND	7.04	6.50	5.83	1.26	2.22	6.04	ND	5.09
124689	0.79	1.35	ND	ND	ND	1.67	ND	1.93	ND	ND	ND	0.16
123467	7.37	7.92	ND	9.56	6.22	17.6	12.9	10.2	8.58	11.0	15.3	16.2
123478	4.28	32.5	13.0	15.7	11.8	ND	13.4	9.48	30.2	10.9	19.5	15.6
123678	5.64	13.3	14.1	15.1	10.5	10.5	9.89	5.30	11.6	10.2	9.43	ND
123479	ND	ND	ND	ND	ND	8.37	ND	ND	ND	ND	ND	ND
123469	ND	ND	ND	ND	ND	ND	ND	5.92	ND	ND	ND	ND
123689	8.33	5.98	ND	2.12	5.90	7.31	6.27	5.92	5.59	ND	9.32	5.59
234678	9.54	ND	9.36	11.1	8.21	5.06	8.67	8.15	10.6	9.08	17.3	7.91
123789	6.09	7.67	20.3	2.38	2.48	11.6	5.34	7.89	3.38	10.9	ND	7.07

Table 7. HxCDF isomer distribution of 2007 year soil, %.

Position	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	SB
123468	11.8	7.30	10.5	13.6	11.0	11.0	11.5	10.9	8.66	9.94	10.4	13.1
134678	29.5	20.8	26.6	33.3	35.3	26.9	29.1	28.9	27.7	24.9	26.3	30.3
134679	ND	0.96	1.73	6.54	2.10	1.87	1.97	1.66	1.08	1.46	2.20	2.79
124679	6.53	3.37	4.38	6.27	ND	5.48	4.06	4.07	4.18	2.19	4.23	3.42
124689	1.56	1.74	2.03	ND	2.73	1.61	0.39	0.46	2.05	2.03	3.51	1.18
123467	12.1	9.33	12.8	13.8	4.99	12.6	11.9	13.2	9.66	14.8	9.44	11.8
123478	11.2	22.4	7.16	ND	8.75	10.7	13.4	13.0	4.23	13.1	9.48	13.5
123678	11.0	11.2	10.5	11.3	9.89	10.8	10.9	11.5	8.63	9.42	10.3	11.0
123479	2.89	2.97	3.56	ND	6.10	1.98	3.27	3.34	5.97	4.75	3.58	2.78
123469	1.23	1.55	1.44	2.61	ND	1.51	1.19	1.53	2.55	0.85	0.61	1.42
123689	2.97	4.17	5.00	ND	ND	4.31	3.48	1.06	7.71	4.42	4.35	1.95
234678	6.53	9.52	7.32	12.6	12.7	6.74	5.67	6.61	12.0	9.20	10.4	4.41
123789	2.78	4.68	6.98	ND	6.50	4.58	3.29	3.83	5.58	2.97	5.21	2.47

Table 8. HxCDF isomer distribution of 2008 year soil, %.

Position	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	SB
123468	10.9	9.80	9.35	9.70	7.05	9.68	10.1	8.67	8.07	8.76	9.40	8.73
134678	27.2	24.6	26.1	28.0	27.4	24.3	26.1	22.8	26.8	26.3	27.3	24.5
134679	2.14	1.39	1.82	1.29	ND	1.16	1.61	ND	1.12	1.60	0.77	1.54
124679	4.48	3.89	3.94	3.82	5.47	5.39	5.53	7.98	4.61	3.11	5.45	4.08
124689	2.00	1.83	0.41	1.25	1.54	3.13	1.86	5.71	1.70	1.63	2.19	1.36
123467	12.1	11.3	11.6	11.8	12.2	13.8	13.2	13.5	13.5	13.2	14.7	12.5
123478	9.74	15.7	8.44	9.90	9.50	8.97	8.32	10.0	8.47	9.91	7.29	18.7
123678	9.78	10.6	9.86	10.3	9.49	8.35	10.6	10.1	9.49	10.7	8.40	10.6
123479	1.99	2.50	2.78	2.94	2.85	2.68	2.24	4.31	5.46	4.90	3.90	ND
123469	2.12	1.38	1.23	1.58	1.57	2.65	1.39	1.14	1.10	1.77	1.67	0.96
123689	3.13	4.25	4.68	5.36	7.52	4.61	5.54	4.89	8.34	5.44	6.11	4.72
234678	10.6	8.56	9.08	10.2	10.4	10.2	10.1	7.20	7.92	8.84	8.65	7.96
123789	3.74	4.19	10.7	3.85	5.00	5.07	3.49	3.65	3.35	3.89	4.11	4.40

Table 9. HxCDF isomer distribution of 2009 year soil, %.

Position	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	SB	Ash	Gas
123468	11.4	9.34	9.76	10.7	8.78	8.95	10.1	7.61	8.17	9.36	5.99	10.3	8.62	9.83
134678	26.5	25.1	26.1	28.7	25.4	25.5	27.1	23.6	24.5	25.7	18.6	28.3	20.2	29.9
134679	1.66	1.45	1.03	1.90	1.56	1.63	ND	1.06	1.65	1.80	ND	2.14	1.59	1.88
124679	3.39	3.50	2.96	3.88	4.07	2.23	3.12	2.74	5.82	4.00	2.94	4.45	3.28	3.32
124689	1.83	1.87	1.30	1.85	1.70	0.67	1.22	1.55	ND	ND	1.34	ND	2.12	1.56
123467	13.4	11.1	13.3	10.2	11.8	11.8	13.7	13.5	10.8	12.4	33.8	18.2	13.4	10.4
123478	10.4	14.9	9.72	9.70	12.3	11.8	9.95	16.3	11.9	12.0	8.78	ND	14.0	9.66
123678	10.5	11.2	11.1	10.2	10.3	10.8	11.5	11.4	11.2	10.5	14.9	12.5	13.2	11.2
123479	2.14	2.22	2.37	2.73	2.44	2.70	2.96	2.33	4.20	3.88	2.60	2.74	1.52	1.27
123469	2.02	1.74	1.88	2.04	1.44	1.45	ND	1.14	1.01	1.75	1.58	1.27	2.73	1.94
123689	2.85	3.74	3.66	4.23	5.65	6.28	5.01	3.53	7.68	5.36	3.87	2.67	2.49	3.07
234678	11.3	9.86	10.8	10.5	10.5	9.77	12.0	10.6	9.84	9.19	5.67	14.5	13.5	13.0
123789	2.62	3.96	6.03	3.28	4.08	6.46	3.34	4.60	3.16	4.01	ND	3.09	3.33	2.97

Table 10. HxCDF isomer distribution of 2010 year soil, fly ash and stack gas of MWI, %.

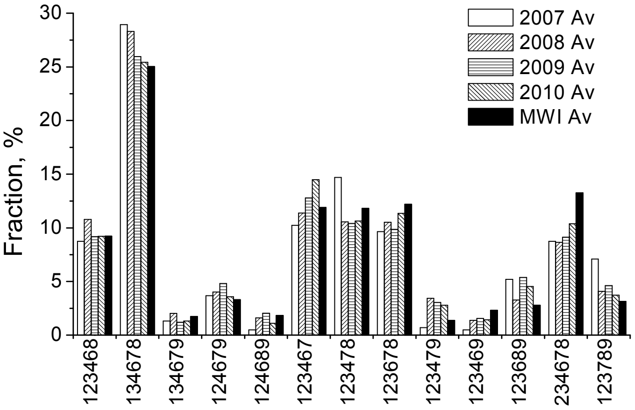

Fig. 9. HxCDF isomer pattern of soil and MWI samples (Av, Average).

The PCA result of HxCDFs isomer profile is shown in Fig.10. Two principal components are extracted from the analyzed 13 isomers. Although component 1 and component 2 can only explain 32.72% of samples' information, while it still can give some useful information for investigating the relation among soils and MWI emission by PCA of isomer profile. In Fig.10, it is observed that 2007 soil spots locate in a large scale, apart from each other, and far away fly ash and stack gas, which means significant difference between 2007 soils and MWI emission. Other year soils have slight trend of assemblage together, meanwhile, become closer to the location of fly ash and stack gas. The points of 2008 and 2010 survey soils are closer to MWI than the sites of 2009 soils, and the group of 2010 survey soils has comparatively closest relation with MWI emission in the PCA plot. This is in line with the variation of PCDD/Fs concentration, and the HxCDF isomer profile also become more likely with MWI emission with year's past.

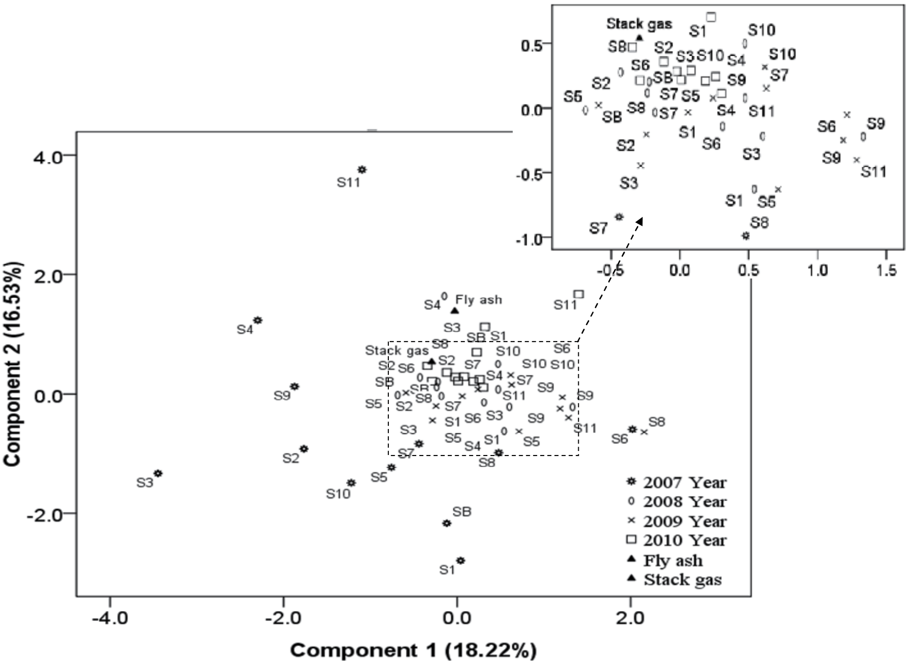

Fig. 10. PCA plot of HxCDF isomers distribution.

4. Conclusion and future work

In the present study, it was observed that dioxin level varied in the analyzed four years, generally, the average level increased after this MWI operation started, as long as just a slight increase in the background samples. The most significant variation is detected in the sites close to this plant, and accounting for the relation of variation and distance away the stack, a limited region near MWI (approx. 500 m) is assumed to be under the influence of PCDD/Fs emission from this MWI. By the PCA of PCDD/Fs homologue pattern and HxCDF isomer profile, PCDD/Fs characteristic distribution in soil became more and more similar with the character of MWI emission. The present PCDD/Fs concentration in this region is in the normal level by the comparison with other studies over the worldwide. In

China, the monitor of dioxin emission for every plant by the environment protection bureau is just one time per year (three samples for a time), so that the information of daily emission is unknown. Some incinerators would release higher concentration of pollutants, which would cause the pollution in the vicinity of plants. Thus, a comprehensive supervised system and more stringent emission limit standard should be established. This tracking monitoring study will be continued in the future years, and the air samples also would be collected and determined to better clarify the environmental impact of waste incinerators. At present, the baseline survey of PCDD/Fs in vicinity soil must be done and noted before the operation of new incinerators according to latest Chinese regulations. The baseline will be used to the comparison with the vale of PCDD/Fs in soil years later, which is basic method to assess the environmental impact of plant.

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2

Research for Investigating and Managing Soil Contamination Caused by Winter Maintenance in Cold Regions

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1. Introduction

In the north temperate and arctic zones, large amounts of de-icing chemicals are used during the frozen season for winter maintenance of highways, roads, airports and other surface areas. The transport sector, and civil aviation in particular, has become a major industry and is one of the fastest growing sectors of the world economy (Janic, 1999). Following an increased concern for the environment in general (Lutz, E. & Munasinghe, M. 1994; Willems, 1994; Jackson, 2010; EEA, 2009), pollution from airports and roads (EPA, 1995) has received more attention. It is also considered one of the contamination threats to soil according to the proposed EU Soil Framework Directive introduced by the European Commission in 2006 (COM (2006) 232; Tóth et al., 2008). In this chapter we will discuss various processes that need to be considered from the source to the recipient, which could be the groundwater or surface waters, and how these are affected by cold climate (winter frost). The source will be related to road network or runways, but can potentially be both a line source if no collection or surface drainage is in place, or can constitute a point source. In cases where road or runway runoff is collected in retention systems with subsequent infiltration into local soils or release to surface waters such as creeks or rivers it could potentially be a significant point source. Often there are no legal limitations on total amounts of salt that can be applied on roads and highways, hence there is no control of their release to the environment. Climate change may cause increased fluctuations about the freezing point which is a condition which increases the use of salts (French et al., 2010). Hence these chemicals may constitute a long term threat to soils in areas with frozen conditions in winter. The second process is infiltration into frozen, partially frozen or unfrozen soils, depending on state of soil and snow fall in late autumn/early winter. Frozen soils may create impermeable surfaces and highly affect hydrological conditions and in particular boundary conditions for unsaturated flow. Further, the flow and transport in the unsaturated zone is affected by soil physical and bio-geo-chemical heterogeneities and in cold climate, high temporal variability in degradation potential due to low temperatures during winter and snowmelt. If or when chemicals arrive at the groundwater level heterogeneous conditions continue to influence the fate of de-icing chemicals but the general mechanisms that apply have been widely documented elsewhere. In the next sections these processes and how they can be measured and modeled will be presented and we suggest how this knowledge can be used in planning of management strategies. The focus of this chapter is on processes near the surface and in the unsaturated zone. In the conclusions we discuss challenges still unresolved.

2. De-icing chemicals

There are two types of de-icing chemicals; non-degradable inorganic salts and degradable organic compounds. Table 1 shows de-icing chemicals and their associated chemical oxygen demand in the case of complete degradation. For the inorganic salts, the only means of reduction is dilution.

Group	Name	Chemical formula	Chemical oxygen demand (COD)[1)]
Inorganic salts	Sodium Chloride	$NaCl$	0
	Magnesium Chloride	$MgCl_2$	0
	Potassium Chloride	KCl	0
Organic salts	Potassium Formate	$KCOOH$	0.35
	Sodium Formate	$NaCOOH$	0.35
	Calcium Magnesium Acetate	$CaMg(CH_3COO)_4$	4.28
	Sodium Magnesium Acetate	$NaMg(CH_3COO)_3$	3.21
	Potassium Acetate	KCH_3COO	1.07
	Sodium Acetate	$NaCH_3COO$	1.07
Others	Propylene Glycol	CH_3CHOCH_2OH	1.69
	Ethylene Glycol	$HOCH_2CH_2OH$	1.29
	Diethylene Glycol	$(HOCH_2CH_2)_2O$	
	Fructose/glucose/Sodium Chloride	$C_6H_{12}O_6$/ $C_6H_{12}O_6$ /$NaCl$	
	Urea	$(H_2N)_2CO$	2.13[2)]

Table 1. Most commonly used de-icing chemicals. [1)] Chemical oxygen demand (COD) mg/l based on standard stoichiometrical calculations assuming complete degradation [2)] assuming reduction to NH_4.

2.1 Inorganic salts

Some examples of total use of salts on Norwegian national roads per winter season are given in Table 2. The average salt consumption in 2009/2010 was 14 tons per km high priority roads.

Negative consequences of increased salt concentration along roads have been documented (e.g. Nystén and Suokko, 1998; Oberts et al., 2000). Howard & Maier (2007) simulated increased NaCl concentrations in groundwater due to urbanisation near Lake Ontario. According to their Visual Modflow simulations, maximum concentrations of 5000 mg

NaCl/l were predicted near roads with an annual use of 20-250 tons NaCl per km road (dual to multiple lane motorways), and that stabilisation occurred after 700 years. The consequences for the environment can be groundwater of insufficient quality for drinking water. Direct or indirect release to lakes can give hypolimnetic conditions and prevention of biannual full circulation with reduced oxygen levels (Bækken et al., 2006). Release into creeks can give negative effects to biologic systems (Meland, 2010).

Winter season	NaCl (tons)	Sand(tons)
2005/2006	166,000	435,000
2006/2007	137,000	386,000
2007/2008	160,000	484,000
2008/2009	200,000	443,000
2009/2010	201,000	417,000

Table 2. Total consumption of road salts and sand on Norwegian national roads (the Norwegian public roads administration, www.vegvesenet.no).

2.2 Organic de-icing chemicals

Organic salts such as Potassium Formate, Potassium Acetate and Calcium Magnesium Acetate are used on runways and some roads (US EPA, Amundsen et al., 2008). These chemicals are more costly than the inorganic salts, but are used because they are degradable and less corrosive. Normally these chemicals are also released directly into the ground next to the road or runways.

Propylene glycol, Ethylene glycol and Diethylene glycol are the main constituents of de-icing chemicals used to keep planes and other vehicle surfaces free of ice. The de-icing at airport takes place on special de-icing platforms which normally collect the surplus chemicals with subsequent re-cycling or treatment in treatment plants (Øvstedal & Wejden, 2007). Estimations from Oslo airport, Gardermoen, suggest that 80% is collected at the de-icing platforms, about 10% is released to the local environment at take-off, and 10% leaves with the planes (Øvstedal & Wejden, 2007). Still many airports in the world do not collect the de-icing chemicals and they are released into the local environment (US EPA).

The treatment of water contaminated with organic de-icing chemicals is usually based on in-situ or off-situ aerobic and/or anaerobic degradation. The tested techniques vary from constructed wetlands, reed beds, constructed soil filters (Roseth and Bjørnstad, 1998; Roseth et al., 1998) natural soil profiles (French et al., 2001, Jaesche et al., 2006) to more conventional wastewater treatment plants (Rusten et al., 1999). The chemicals are supplied during the frozen season which may vary geographically but falls roughly between October to April. The objective of using de-icing chemicals is to reduce the freezing point of water, hence water will remain in a fluid state below 0°C and can infiltrate into the ground as long as there are open pores. Water containing de-icing chemicals may therefore infiltrate the ground prior to the main snow melting period. The preferential melt-out of de-icing chemicals was shown by French & van der Zee (1999) as also shown for other chemicals stored in snow (Johannesen and Henriksen, 1978). Although the organic de-icing chemicals are degradable and the top soil hosts large amounts of bacteria and fungi, the temperatures are negative or close to zero, and there is little degradation during this period. Half lives of Propylene Glycol and Acetate under field conditions throughout melting period and into the summer ranged between 15-46 days (French et al., 2001). Degradation rates of other airport chemicals, as well as the increase of rates caused by optimizing the C:N:P ratio are reported in French et al. (2002). Although degradability of de-icing chemicals in general is positive for the environment, negative consequences can be anaerobic conditions. Field experiments (French et al., 2001) showed an increase in manganese concentration when propylene glycol and Potassium acetate was supplied to the ground surface even in the unsaturated zone, indicating oxygen limitation. On-going research (French et al., 2009) suggests that nitrate could increase remediation and improve redox conditions in local soils.

3. Non-invasive versus destructive methods for soil characterisation

The theoretical considerations concerning spatial variability, described in the next section requires that we have some knowledge about 1) the geostatistics of the hydrogeological properties, and 2) the scale of the contaminant source or plume relative to this variability.

3.1 Non-uniform infiltration

The boundary conditions influencing the flow and transport during snowmelt are characterized by ground frost and the formation of impermeable ice on the ground surface which redistributes melt water during the snowmelt period (Fig. 1). The ice-cover often inhibits infiltration in sediments with otherwise high infiltration capacity (sandy aquifers). As a result, a large amount of melt water collects in depressions or becomes surface runoff. According to Baker & Spaans (1997); Derby & Knighton (1997); Johnsson & Lundin (1991), infiltration during snowmelt often occurs as focused recharge in local depressions on the surface.

Monitoring water accumulation in snow and also the infiltration below the snow cover is a challenge. The traditional but laboursome way of collecting information about snow cover is to measure snow depths along a transect and estimate the snow water equivalent (SWE) by weighing the snow column. The advantage is that one gets a spatial coverage, but only for single times, also the method is destructive. Snow pillow measurements involves placing a logged scale beneath the snow cover, hence direct measurement of SWE above the snow pillow, this method prevents infiltration and is only representative of one location, also it may give wrong values when ice crusts are formed in the snow which reduces the weight load on the scale. Remote sensing using natural emissions of gamma rays and micro waves has become a common method for mapping snow storage over larger areas (Glynn et al., 1988; Durand et al., 2008; Schaffhauser et al., 2008), but also local measurements can be conducted using this principle (Campbell scientific; Bland et al., 1997).

A snow lysimeter is a method where meltwater is collected via drain pipes from trays below the snow cover, and volumes and quality measured. Hence destructive in the way that water is prevented from infiltrating the ground. Variations in release of water from a melting snowpack was documented by French & van der Zee (1999) by collecting meltwater from snow lysimeters placed beneath a melting snow cover. Despite a fairly uniform snow depth over the monitored area (a few square meters), the total melt volumes varied from nearly 0 to 200% of expected values based on the snow water equivalents measured prior to snowmelt. To further characterise the infiltration pattern in the soil, French & Binley (2004) installed electrodes near the surface and monitored changes in electrical resistivity of the soil volume, which indirectly reflected changes in water contents during snowmelt. Figure 2, shows the spatio-temporal variability of electrical resistivity observed in a horizontal plane during this experiment. This may cause higher velocities through the unsaturated zone than during evenly distributed infiltration on the surface, hence causing less than optimal conditions for degradation of pollutants. Preferential meltout of chemicals and subsequent redistribution of meltwater may also cause concentrations in soils next to the impermeable covers which are higher than expected from a pure mass-balance point of view. In addition to the temporary and variable surface conditions created by frost and snow, impermeable surfaces, membranes and other installations near the soil surface may highly affect the infiltration and flow pattern in the unsaturated zone next to roads and runways (e.g. Apul et al., 2007).

Fig. 1. Surface conditions at Oslo airport, Gardermoen, near the end of snowmelt, showing patchiness of snow and ponding of melt water due to soil frost.

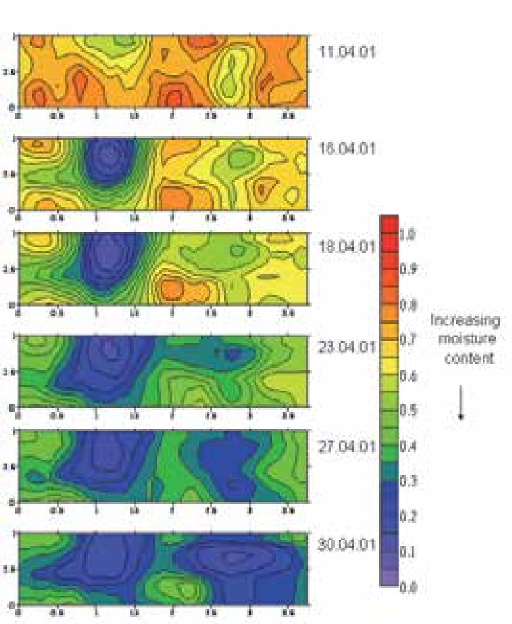

Fig. 2. Variable infiltration, increased moisture content as blue areas, observed as reduced electrical resistivities near the surface of a soil below a melting snow cover (modified from French & Binley, 2004).

3.2 Characterisation of soil heterogeneity

Conventional techniques, such as soil sampling and core drillings provide more or less disturbed samples on which one can measure porosity, unsaturated hydraulic conductivity and Pf curve (if undisturbed) or grain size distribution and water content on disturbed samples. The Pf curve describes the relationship between soil suction and soil water content, this curve determines the unsaturated hydraulic conductivity (ref). Well established pedo-transfer functions such as Hazen's equation (ref.) can be used to calculate hydrogeological parameters such as the hydraulic conductivity. The samples can also be used for bio-geo-chemical characterisation interactions between contaminants and soil. A number of spatially collected samples can then be used to establish geostatistical properties of the different hydrogeological parameters (discussed in the next section). More recently advanced direct push technology, which provides opportunities for in-situ measurements e.g. of hydraulic conductivity by the use of specialised probes at the end of the direct push probe. The disadvantage of these methods alone is that they are destructive, time consuming, expensive; and do not give a continuous image of the subsurface. Deep geophysical exploration has been around since the beginning of the last century, and is a common method for geological characterization in oil exploration, mapping of lithostratigraphy, fracture patterns in bedrock and is described in several text books (e.g. Kearey & Brooks, 2002). In the last couple of decades geophysical techniques such as those described in Table 3 have become more common for hydrogeological applications, for further reading see e.g. Regli, et al, 2002; Hubbard & Rubin, 2000, Kowalsky et al., 2001; Rea and Knight, 1998; Rubin & Hubbard, 2005; Veerecken et al., 2006.

As an example we discuss briefly the principle of the electrical resistivity method. The electrical properties of soils are a function of the soil type, water content, soil temperature and ion content of the soil water. Measurements of soil bulk electrical resistivity are most commonly conducted by placing a set of electrodes in the ground along a line on the surface or in vertical boreholes. By inserting a known current and measuring the resultant voltage consecutively over the set of electrodes, one can after an inversion of the collected data obtain an image of the distribution of electrical resistivities in the soil volume next to the electrodes (see eg. Reynolds, 1997). A single measurement may reveal geological features of the subsurface, while the comparison of images taken at different times (time-lapse measurements) can help quantify spatial and temporal variability caused by changes in water (Daily et al., 1992) and ionic contents.

The advantage of geophysical techniques over the more conventional and invasive techniques is that they are non-destructive and provide continuous images of the subsurface. The challenge of geophysical methods however is the ambiguity of their interpretation. The non-invasive geophysical methods map zones or layers of different physical characteristics (Table 3). The interpretation of such data requires that the data is run through an inversion code which basically "suggests" a likely distribution of the specific geophysical responses in a 1, 2 or 3D space. The results are optimised with respect to measurements conducted on the surface or in boreholes. Forward and inverse modelling of the system that is being studied is required for optimising the configuration of measurements, this technique can also be used in a stochastic framework in order to include uncertainty and coupling to soft and hard data for hydrogeological characterisation (Rubin and Hubbard, 2005). Another recent development to reduce the non-uniqueness of the interpretation is to combine different geophysical data sets collected at the same location and time through joint inversion (e.g. Gallado & Meju, 2004; Linde et al., 2006). However combining measurements from geophysical techniques and ground truthing (bio-geo-chemical data) is still required for more complete and accurate characterisation of spatial variability of hydrogeological parameters such as porosity, hydraulic conductivity and contaminant concentration.

Method	**Sensitive to**	**Procedure**	**Can represent**	**Example references**
Seismic refraction/reflection	P-wave velocity and reflectivity	Sound wave, Sound source and set of geophones	Top of bedrock, lithology, water table, faults	Steeples, 2005;
Ground penetrating radar	Dielectric constants	Electromagnetic waves, Transmitter and receiver antennas,	Lithology, water table, water contents	Annan, 2005; Farmani et al., 2007
Electrical resistvity	Electrical resistivity	Electrical current injected and voltage measurements, usually electrodes in the ground	Lithology and zonation, water table, contaminant plumes, water contents	Binley & Kemna, 2005; Daniels et al., 2005;Kemna et al., 2006; Godio & Naldi, 2003
Electro-magnetic	Electrical resistivity	alternating or pulsed current through long wires or coils	Zonation, water table, contaminant plumes, water contents	Everett & Meju, 2005;
Induced polarisation	Chargeability	Same as for electrical resistivity, but non-polarisable electrodes	pore-fluid conductivity and grain-surface polarisation – can be used with ER data to separate lithological units	Binley and Kemna, 2005; Kemna et al., 2006; Vanhala et al., 1992
Self potential	Streaming or Electrochemical potential	Passive voltage measurements, non-polarisable electrodes	Zones with concentrated flow, zones with high degradation	Naudet et al, 2003; Revil et al., 2006; Arora et al., 2007

Table 3. Geophysical methods for soil physical and hydrogeological characterization.

3.3 Characterisation of contaminant plumes

Solute transport, which is basic to natural attenuation processes, requires new measurement techniques to provide spatial distributions and internal spatial characteristics as current modelling of these phenomena is conditioned by availability of experimental data.

Traditional monitoring techniques may neither capture the contaminant distribution nor their removal rates sufficiently (Aagaard et al., 2004). Subsurface characterization of contaminant distribution over large scales is challenging, since the contaminants may have moved erratically through the profile as illustrated in Figure 3, and point samples as provided with conventional sampling techniques (as discussed above) may not provide a representative measurement of the situation. The most common measurement technique for monitoring contaminant transport in the unsaturated zone is sampling of soil water through suction cups. These consist of a porous material such as ceramics or an inert material e.g. Teflon and quartz with a pore size small enough to ensure contact between the filter and the soil. An example of such a system is the experimental lysimeter trench at Moreppen near Oslo airport, Gardermoen, Norway (French et al., 1994). This experimental site has more than 100 Prenart suctions cups and various other soil physical measurements. It was constructed to conduct controlled experiments of transport of Propylene glycol and Potassium Acetate during snowmelt. Several studies were carried out at the same site in order to examine the hydrogeological properties in the unsaturated and saturated zones and the transport processes.

Contaminants may, depending on their chemical properties, affect the geophysical signature of the soil. Salts will increase the electrical conductivity (EC) of the pore fluid, while hydrocarbons will have the opposite effect. The organic and inorganic de-icing salts will reduce the electrical resistivity of the soils, while Propylene glycol will not affect the electrical conductivity of the pore fluid. Electrical and electromagnetic methods are widely applied for soil mapping and detecting of contaminated plume. Over the last decade new geophysical methods such as induced polarisation (e.g. Godio and Naldi, 2003; Slater & Mansoor, 2006), electromagnetics, GPR, micro-sesimics and self potential (Naudet et al., 2003; Arora et al, 2007) have been explored as methods for exploring contaminated sites. Low frequency electromagnetic (EM) methods are usually adopted for fast mapping and preliminary assessment of the aerial extent of the potentially contaminated land. A qualitative image of the soil mineralization, due to degradation of hydrocarbons, could be inferred by integration of resistivity and induced polarisation data (e.g. Godio and Naldi, 2003, Slater et al. 2006). Electrical Resistivity Tomography (ERT) is a powerful tool for investigating pore fluid properties (Olsen at al., 1999; Kemna et al., 2000; Depountis et al., 2001; Damanesco and Fratta, 2006;) as shown in laboratory experiments (Comina et al., 2005) and for solute transport in undisturbed soil columns (Binley et al., 1996) and field sites (Slater et al., 2000; French et al., 2002, Binley et al. 2005). How to estimate hydrogeophysical parameter is still one of the major challenges, state-of the art knowledge is described by Linde et al., (2006). Another challenge for combined interpretation of geophysical and point measurements is that the support scale of different methods varies; hence a statistical framework is required for joint interpretation.

4. Modelling implications

As evidence shows, the subsoil is in general heterogeneous (or spatially variable), and often this heterogeneity is partly irregular. This irregular variation has been the motivation to consider soil as an intrinsically random material, i.e., as a material that can only be described statistically. This assumption has resulted in a large body of literature (Bellin et al., 1993; Dagan, 1997; Keijzer et al., 1999; Janssen et al., 2006; Cirpka, O.A., P.K. Kitanidis, 2000; Fiori et al., 2002), that is still actively being developed and is quite mathematically inclined:

stochastic groundwater hydrology and contaminant hydrology. For the present purpose, it is not useful to emphasize the mathematics, although some of it cannot be avoided. Rather, emphasis is given to illustrate the effects of heterogeneity and to give an impression of what that means for decision making.

4.1 Stochastic hydrology

The mentioned stochastic approach to contaminant hydrology (that focuses on the transport behaviour and fate of soil and groundwater contaminants) implies that particular properties are considered to be random space functions (RSF): they vary irregularly in the horizontal plane and with depth. However, they do not do so completely randomly: due to the genesis of layering, and soil horizons, patterns of large and small values can be observed. These patterns are described by autocovariance functions. Examples of strongly variable properties are the hydraulic conductivity, and for the water unsaturated zone also the soil water retention function (Van der Zee & Boesten, 1991). Much research has established that the hydraulic conductivity, and the scaling parameter in this function that also is used to describe water retention, are well described with a lognormal distribution. Also for several important properties that control the adsorption behaviour of contaminants, the lognormal distribution appeared to be reasonable (Van der Zee & Van Riemsdijk, 1987, 1988; Boekhold & Van der Zee, 1991).

Randomness can have a large effect on flow and transport (Rode et al., 2010). Basically, this effect is (i) a more irregular transport behaviour of the contaminants, and (ii) a larger uncertainty about this behaviour. To give an impression of how heterogeneity affects the transport pattern, Figure 3 shows the leaching behaviour of both inert chloride and degrading de-icing chemical at Oslo Airport, Gardermoen (French et al., 2001). We observe a very irregular 'plume' of contaminant. A problem with such complex patterns is that they are difficult to communicate, other than by sending a picture.

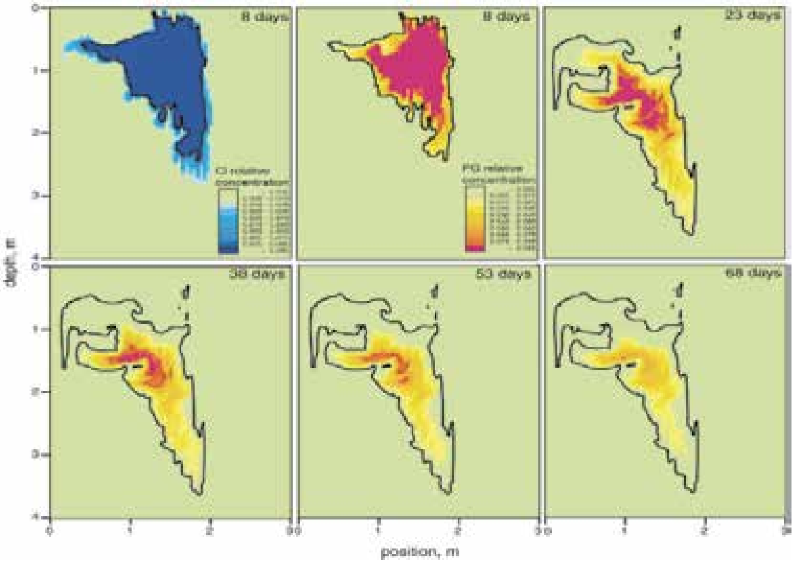

Fig. 3 The erratic pattern of chloride and propylene glycol transport in a heterogeneous soil representative of the Moreppen site at Gardermoen (French et al., 2001).

In addition, we have to deal with uncertainty, because the pattern if the contaminant were released on a spot a few meters away, might look similarly irregular but not exactly the same! It might be slightly deeper or shallower, moved to the left or right, and have another shape. Whereas for contaminated sites, it may be meaningful to know the exact pattern, for making predictions, the precise shape of the plume becomes less important: a calculation for another spot would look always more or less different. For this reason, we need more robust measures to quantify the transport behaviour, than the pictures of Figure 3.

4.2 Spatial moments

To this aim, we explain the moment theory, with which such quantification is feasible at different levels of detail. Using this moment theory, it is feasible to illustrate the profound impact of spatial variability on contaminant transport in terms of pollution front, breakthrough time, and of course remediation efficiency.

If soil and aquifer properties are spatially variable, two limiting situations may arise. These two limiting situations are related with those of point source pollution and of diffuse source pollution, respectively. The point source pollution problem can be defined as the situation where the source is relatively small (or of similar order of magnitude) compared with the scale of (random) heterogeneity. In that case, the source of contaminants might be visualized as being in a spot of large or of small hydraulic conductivity. In the first case, contaminants may readily spread in the environment, whereas in the latter case, this occurs slowly. For the point source pollution event, it is difficult to predict whether the source will be at in contact with the fast or the slow route of transport, which leads to high uncertainty. In the diffuse source pollution case, contaminants enter the soil or aquifer over a large surface, and both fast and slow transport routes are 'sampled'. In that case, uncertainty is much smaller, and heterogeneity leads to large spatial variability of fluxes. This distinction of two limiting situations is illustrative, but in view of the nested scales of heterogeneity of soil and aquifers, many real situations will be somewhere in between.

Fig. 4. Photo of a heterogeneous soil profile.

It is clear from Fig. 4, that the thickness and intensity of soil horizons varies in the horizontal plane. As soil genesis processes involve longer time periods, spatial variability must be due to persistent differences in soil composition and do not necessarily result from differences in 'input' at the soil surface.

For the two limiting cases mentioned above, the 'diffuse' pollution case is called an ergodic situation, because 'all' heterogeneity is sampled simultaneously. This leads to a distributed answer, such as a spatially variable contaminant plume or breakthrough curve. If these answers are measured or calculated at another place, they do not significantly change. Hence uncertainty is limited.

However, the distributed answer is complicated and difficult to communicate to others who cannot see the picture. Moreover, it is commonly too detailed for e.g. management decisions. Therefore, it is appropriate to consider robust measures of the results, which focus on the major issues. Useful examples of such robust measures are found in the theory of moments. We will give the definition of these moments in terms of spatial moments (representing the situation at one instant in time), but temporal moments (at one particular location, plane or volume) are equally feasible. The spatial moments of a property P, where the space coordinate is x, are given by

The zeroth' moment, or the mass of the distribution:

$$M_0 = \int_{-\infty}^{\infty} f_P dP \tag{1}$$

The first moment, or the mean of the distribution:

$$M_1 = \frac{1}{M_0} \int_{-\infty}^{\infty} P f_P dP \tag{2}$$

The second central moment, or the variance of the distribution:

$$M_2^c = \frac{1}{M_0} \int_{-\infty}^{\infty} \left(P - M_1\right)^2 f_P dP \tag{3}$$

For a contaminant plume as shown in Figure 3, the zeroth' moment is equal to the amount of dissolved chemical in the contaminant plume, if P is equal to the position x, and the function fx represents the spatial distribution of the contaminant concentration multiplied with the water-filled porosity. The first moment represents the mean position (in direction x) of the contaminant distribution and the second central moment represents the width of the zone over which the concentration distribution occurs. Whereas we illustrated the moments where P is equal to position, many other properties can be chosen. Which to choose depends on the primary interest, but examples are the concentration or mass of contaminant, the quantity or fraction of contaminant leached beyond a reference plane, such as groundwater level and so on. In principle, the transport problem for de-icing chemicals is a very complicated one, even in one direction such as depth. The reason for that is that a complex of interactions is affecting this transport. Examples are infiltration conditions, ad/desorption, microbial degradation according to different kinetics (1st, Monod, 0th order), and a host of different redox-sensitive components that may affect the degradation rate (Beltman et al., 1996; Keijzer et al., 1998) may occur, depending on ambient conditions as well as the influx of degrading substrate (continuous, instantaneous). To give an impression of the complexity, we provide graphs in Figure 5, that reveal that the concentration pattern may be completely different from what we are used to, and that the transport process is quite irregular in space.

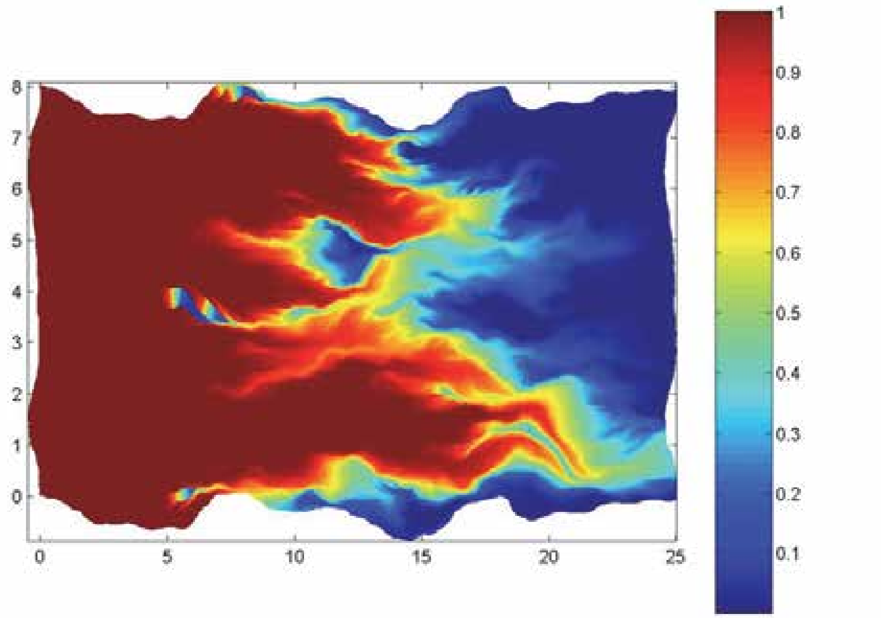

Fig. 5. Computationally determined map of concentrations of an electronacceptor (EA) such as oxygen or nitrate with scaled concentrations ranging from 0 to 1. The EA enters the aquifer from the left and moves towards the right, and is used by micro-organisms to degrade initially resident gasoline that contaminates the aquifer. Computationally, this situation is quite demanding to solve, and a streamline dependent spatial discretization was needed to suppress numerical dispersion (Janssen et al., 2006).

Figure 6 gives a representation of the behaviour of Figure 5, in terms of concentration distributions in the longitudinal direction. This result implies that the three linked concentration curves for initially resident degradable contaminant G (gasoline, or kerosene, for instance), an incoming (from the left) electron acceptor (EA such as oxygen) C, and resident, growing and decaying bacterial mass M, show patterns that for each time are directly related with each other. What is complicated is that the electron acceptor concentration distribution for all three times is different, and that between time 1 and 2 the injected electron acceptor seems to withdraw to the left again. This seemingly illogical result is purely the consequence of the increasing microbial mass when both substrate (G) and e.g. oxygen are present, as then, the consumption of G suddenly increases and its concentration decreases. As Figure 5 reveals, spatial variability of the hydraulic conductivity may strongly affect the transport of electron acceptor and the related degradation of resident contaminant. Erratic patterns may develop, that are difficult to communicate.

Whereas the complexity of the transport problem is profound, the strength of identifying the right robust features may inspire to equally profound simplifications. Considering the downward transport of a degrading contaminant towards the groundwater level, the mass balance equation for linearly adsorption and 1st order degradation reads:

$$\rho\frac{\partial q}{\partial t}+\theta\frac{\partial c}{\partial t}=\theta D\frac{\partial^2 c}{\partial x^2}-\theta v\frac{\partial c}{\partial x}-k\left(\theta c+\rho q\right) \tag{4}$$

Where q is adsorbed amount, c is concentration, k is degradation rate, v is pore velocity, D is dispersion coefficient, θ is volumetric water fraction, ρ is density.

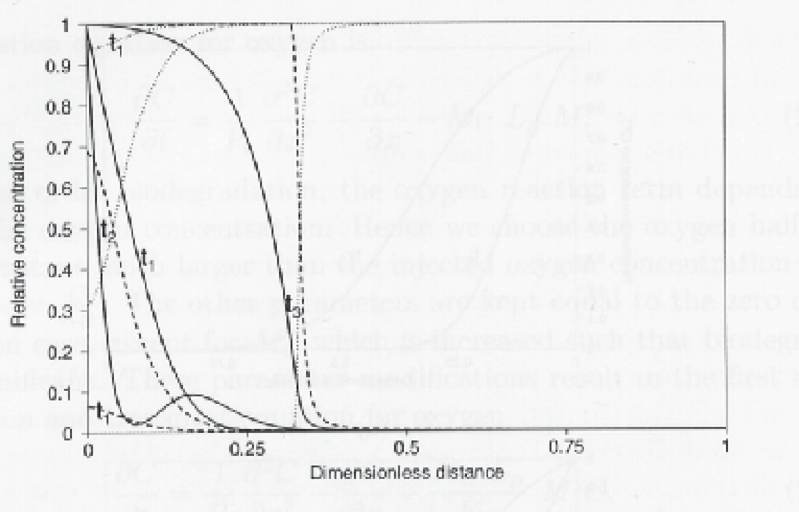

Fig. 6. Concentration distributions for electron acceptor (solid), contaminant (dotted), and microbial biomass, for times increasing in the direction of t_1 to t_3. Concentrations scaled with regard to their maximum concentration.

In essence, according to this transport equation, adsorption/desorption is instantaneous, and the degradation occurs (according to the last term) both in the liquid and in the solid phases. In terms of system properties, physical properties such as the hydraulic conductivity and water retention, control the development in time of the volumetric water fraction (θ) and flow velocity (v). Biogeochemical properties of importance are those related with the adsorption equation, s(c), e.g. affinity coefficient and a sorption maximum, and those related with the degradation rate, e.g. Monod kinetics parameters that describe the degradation rate as a function of the concentrations of contaminant, of electron acceptors or reductors, and microbial activity. Commonly, degradable contaminants are assumed to degrade according to first order kinetics. For the given transport equation, the transport and leaching process can be simulated with a computer model such as PEARL (Leistra et al., 2000). The PEARL model considers one-dimensional transient flow of water in the soil (vadose zone) profile, which is linked to the convection dispersion equation (CDE) for transport of chemicals. Besides that the model has modules for crop growth and energy and water interactions with the atmosphere, it is also possible to account for (monocomponent) adsorption/desorption and first order degradation. The PEARL model(see Beltman et al., 2008) is used in pesticide screening in the EU, for which the FOCUS scenarios have been developed ((FOCUS, 2000).). These scenarios harmonize the soil type, climate and weather variations within the EU for which the assessment of pesticide leaching to groundwater is evaluated, with several aims, among which the need to consider a limited number of modeling permutations, while still enabling comparison of results EU-wide.

5. Implications for management strategies

5.1 Simplified modeling approach

For decision making, we propose to first make an approximate assessment based on several simplifications. Thus, diffusion-dispersion is considered to be of secondary importance and flow is assumed to be steady state (as a first step). For groundwater quality protection, particularly the leached fraction of applied or spilled contaminant is of interest, because that quantity will control the concentration in the annual recharge of the topmost aquifer. For a particular year, both the leached fraction and the recharge (precipitation minus evapotranspiration) may be difficult to predict, which is an example of lack of ergodicity in time. However, if the interest is primarily for long term predictions as is the case with de-icing chemicals applied every winter, rather than for incidental spill events, then the uncertainty becomes considerably smaller. Regular leaching leads in that case to repetitive concentration jumps in the upper groundwater aquifer, and whether these jumps lead to a gradual building up of concentrations in groundwater, can be analysed according to the method of Beltman et al. (1996) and Van der Zee et al. (2010).

In those papers, we showed how the regular leaching of contaminant can result in a building up of concentrations in groundwater, depending on the capacity of the soil to degrade the contaminant: Figure 7. Whereas Beltman et al. (1996) focused on degradation and transport in an aquifer, Van der Zee et al. (2010) considered leaching from a mixed reservoir analogous of the unsaturated soil. In essence, the mathematical formulation does not depend on whether the first order kinetics of the contaminant transport and removal are due to transport processes or to degradation. Hence, in analogy to the approach of Van der Zee et al. (2010), we can consider an unsaturated soil volume, that looses contaminant to the groundwater. The quantity that is lost, we consider below, but if the lost quantity is equal to M and the volume of water in the unsaturated soil zone equals V, then

$$C = \frac{M}{V}\frac{\left[1-\left(exp\left(-\frac{j\Delta t}{V}\right)\right)^{n}\right]}{1-\left(exp\left(-\frac{j\Delta t}{V}\right)\right)} \tag{5}$$

For the maximum concentrations of the resulting saw tooth pattern of concentrations, where j is the water flux leaching from the unsaturated soil, n is the number of applications of the contaminant, and the time step is for one year. Observe that the minimum concentrations of this pattern are given by C-M/V.

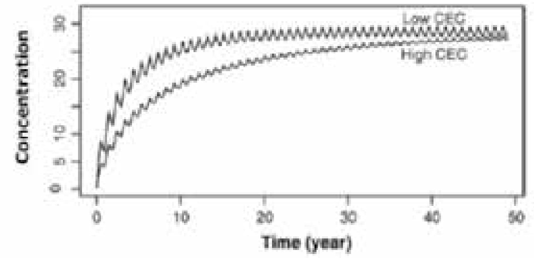

Fig. 7. Saw tooth patterns of concentration for an application of contaminant every year for two different values of CEC (cation exchange capacity), which controls concentration buffering. From: Van der Zee et al. (2010).

For a homogeneous soil, the leached fraction, *F*, according to the Convection Dispersion Equation is given by

$$F = \exp\left[\frac{-\mu R L}{v}\right]; \qquad R = 1 + \frac{\rho}{\theta} K_s \tag{6}$$

if dispersion is ignored, where R is the retardation factor and K_s is the sorption coefficient for linear sorption. In this solution, we recognize the soil physical and atmospheric forcing controlled properties (θ, v), and the contaminant specific properties (K_s, and degradation rate parameter μ). Hence, a leaching vulnerability assessment system for the applicability of de-icing chemicals could involve a water flow and tracer leaching investigation, followed by a contaminant specific leaching investigation. In real soil systems, the various parameters of equation (6) vary in space and time, and this may have a large effect on the leached fraction. Therefore, Van der Zee & Boesten (1991) simulated leaching for the heterogeneous case. It appears that if there is a weak spot in the soil where leaching is relatively large and fast, then this one spot can contaminate a large volume of water up to the level of the water quality standard even if elsewhere no leaching of contaminant occurs. For moderate variability of soil properties, the leaching of contaminant in such a heterogeneous environment still behaves as 'first order degradation and transport', as described by equation (5), but the constants in this equation should be appropriately averaged. How this averaging should be done, focused on the leached fraction, is explained by Van der Zee & Boesten (1991).

Aimed at the problem of contamination with de-icing chemicals, it appears feasible to approach surface runoff also from a partly stochastic approach. Therefore, some first indication of ways to deal with irregular soil surfaces in the case of overland flow are presented using the framework of Appels et al. (2011). Using these approaches, it appears to be possible to give a stochastic analysis of the in situ aquifer bioremediation of degradable contaminants such as Propylene Glycol. Besides illustrating this aspect, many problems and unresolved questions are outstanding. For instance, mostly soil and groundwater formations are assumed to conform to Gaussian spatial structures, but in reality, more complicated structures that are distinctly non-Gaussian, may be more realistic.

5.2 Remediation in heterogeneous soils

Because transport of nonlinear reacting contaminants in spatially variable soils is a very active field of research, many problems are unresolved, yet managers need to make decisions, for instance concerning choice of remediation technique. For this reason, it is appropriate to indicate in what respect management decisions need to take into account this complexity. For instance, the applicability of the still recommended (e.g. by USA, EPA) air sparging as a methodology to purify groundwater that is contaminated with organic biodegradable or volatile contaminants can be judged well on the basis of the physics of this technique.

In air sparging, air is injected below the phreatic groundwater level, with the purpose to strip the aquifer from volatile contaminants, but also to increase the oxygen content in those strata as certain organic contaminants require it to become degraded. Much effort has been devoted to experimentally investigate how air will flow through the aquifer (upwards, as air is much less dense than water): in a narrow, vertical funnel or in a gradually upward broadening cone. The latter occurs if the aquifer material is sufficiently fine textured sand, but finer, loamy or clayey textures cannot well be sparged in view of their air entry values. Engineers were particularly interested in the so called radius of influence (ROI), which is the radius of the supposedly circular cross section of the cone through which air flows at the phreatic groundwater level. In addition, air saturations in this cone are of interest.

A mathematical analysis has been worked out by Van Dijke et al. (1995) and was later refined by Philip (1998). In both cases, relatively simple solutions were obtained, but in both cases for a single cone and for a homogeneous aquifer. In a refinement, Van Dijke & Van der Zee (1998) considered a two layered medium, i.e., air was injected below the groundwater level, in a coarse layer that is overlain by a slightly finer textured layer. The contrast in physical properties of the two layers was very small (hydraulic conductivity differed only by a factor √2, i.e., hardly measurable). Even with such a small contrast in properties, the effect on the sparged area was major, as can be seen from Figure 8. It appears that at the interface between the two layers, the cone rapidly expands to larger radial distances away in a horizontal direction from the injection point, and air saturations rapidly decrease. Important is to note, that these calculations were done for an axially symmetric situation. The practical impact of these results is the following: if the air flow pattern is so sensitive to slight spatial (vertical) variability of the subsoil, for real subsoils with intricate spatial patterns in 3D, this must also be expected and (in view of variability) in an enhanced way. The air flow will be along a path of minimal resistance, and along this path, almost all flow will concentrate. Therefore, although injected air will oxygenate the subsoil, this will be a very inefficient way to do so, as flow is excessively preferential. Moreover, it cannot be well steered, to get the air to flow to a particular part of the subsoil, e.g. the contaminated part. In reality, the flow may follow many possible paths around a contaminated volume.

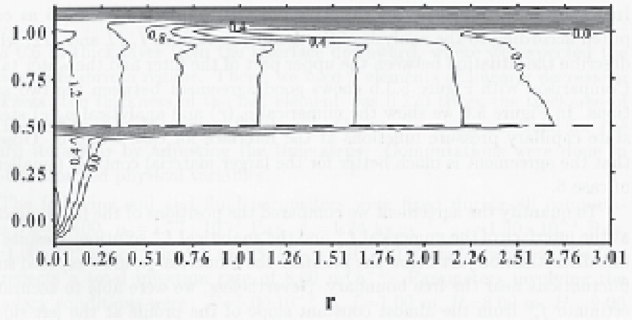

Fig. 8. Iso-concentration lines of air saturation for a two-layered medium with layer interface at dimensionless depth Z. Dimensionless radial distance is r (Van Dijke and Van der Zee, 1998).

In more general terms, spatiotemporal variability leads to uncertainty and therefore some suggestions can be given regarding soil/water contamination decision making under uncertainty, focused at the de-icing chemical contamination context.

6. Guidelines for decision making

Many contaminated sites and polluting surface activities rely on natural or manipulated remediation to remove pollutants in the soil. In order for this approach to be acceptable it is important to have control of: (1) the pollution source, (2) the behaviour of pollutants in soils as influenced by spatial variability of the subsurface, (3) their removal rates (adsorption, degradation, precipitation) and how these can be optimised, and (4) long term effects on the soil system. In the above sections some of these aspects related to contaminants from winter maintenance in cold climate have been discussed, as well as different methods for subsurface characterisation and contaminant transport monitoring. The importance of dealing with heterogeneities in modelling strategy has also been dealt with.

6.1 Legal and political incentives

How contaminants are dealt with at contaminated sites in general, is not only determined by whether the scientific community can overcome present challenges as those discussed in the previous sections, but also within which political and legal framework contaminated site managers work under. Whether the optimal strategies for monitoring and cleanup are chosen depends on the following:

- A correct conceptual understanding of the processes which determine flow and transport of the contaminants, which is the prerequisite for correct choice of modelling tool to assist management at the site.
- The scientific knowledge, do we have the right methods and understanding of the processes to be able to deal with the contamination in a good way? which implies
  - Good methods exists for mapping and monitoring surface and subsurface heterogeneities, at present a combination of destructive and non-invasive techniques are recommended in combination with transport modelling.
  - Robust relationships exist between geophysical properties and hydrogeological properties and sufficient resolution of survey at the site.
  - Robust relationships exist between geophysical properties and contaminant situation in the soil, and sufficient resolution.
  - A sound method for coupling of different methods destructive and non-invasive methods, for quantitative description of contamination levels and remediation efficiency.
  - Appropriate remediation techniques for heterogeneous systems exist.
- Managers' knowledge about the system they are in charge of, the quality depends on:
  - Size of company, in view of available resources made available for such purposes.
  - Type of contaminant – available information about reactive properties
  - Company regulations and policies
- The authorities knowledge about contaminants, survey possibilities, modelling and risk factors, as well as their capacity to follow up contaminated sites and validate that accepted procedures are being followed.
- Legal international and national framework regulating expectations to contaminated sites.

6.2 Cold climate implications

Scientifically many processes are still not well described for low and freezing temperatures, such as physical conditions in freezing soil and how to model these, especially the combination of de-icing chemicals which lower the freezing point in a soil system near 0ºC is complicated. Degradation and biological dynamics as a function of low temperature is generally not well described. Instrumentation may work poorly under frozen conditions, good methods for measuring the total water content in a frozen soil are for instance still lacking. What scale of heterogeneity is relevant during snow melting conditions compared to the thawed situation? Cold climate conditions add extra complications to the contaminant site management, for one; the need for use of de-icing chemicals. Since the use is generally not regulated, there is a great potential for reduced environmental impacts by implementing such limitations. At Oslo airport, Gardermoen, only a certain amount of de-icing chemicals is allowed per winter season. De-icing chemicals that are highly concentrated such as at the de-icing platforms are collected, hence reducing the amount released to the green areas. Since soil temperature has a large effect on surface infiltration patterns, this should be monitored as part of the regular monitoring programme along roads and at airports. Strategies to deal with both frozen and non-frozen conditions during snowmelt must exist, e.g. pump and store contaminated melt water in local depressions for later infiltration when conditions are more optimal, e.g. in summer.

7. Conclusion

This chapter gives an overview of properties of de-icing chemicals and the processes that affect their fate in the unsaturated zone. Inorganic salt concentrations can only be reduced by dilution and is only discussed briefly here, while degrading organic de-icing chemicals are the main focus. Conventional methods of soil and soil water samples are still required for interpretation of emerging technologies such as different geophysical techniques. The importance of heterogeneity of the infiltration process and unsaturated flow is emphasised. The method of spatial moments is an efficient way of describing average movement of contaminants in the unsaturated zone. An ergodic situation, i.e. when the size of the contaminant source is much larger than the scale of soil heterogeneity, makes average assumptions more certain while small plumes in a system of large variability causes great uncertainty. Geophysical methods can be used in time lapse mode to obtain a more continuous impression of the flow and transport processes and is recommended in combination with point measurement for quantification of absolute levels of contaminants. Flow and transport modelling within a stochastic framework is another important complementary technique for optimised management strategies at contaminated sites.

8. Acknowledgement

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3

Soil-Transmitted Helminthic Zoonoses in Humans and Associated Risk Factors

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1. Introduction

The soil is an important route for transmission of numerous human pathogens, including the five major soil-transmitted helminths (STHs), also known as geohelminths, namely: roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), hookworms (*Ancylostoma duodenale* and *Necator americanus*), and threadworm *Strongyloides stercoralis* (Brooker et al., 2006). An estimated one billion people are currently infected with STHs worldwide, particularly in resource-poor settings (WHO, 2011). Although overall mortality due to STH infections is low, morbidity may be significant given the pronounced impact on nutrition, growth, physical fitness, cognitive functions among infected infants, schoolchildren and adults from developing countries (Bethony et al., 2006). In Africa, an estimated 89.9 million children harbor STHs, many of whom are co-infected with two or more STH species (WHO, 2011).

Zoonotic agents, comprising a wide variety of bacteria, viruses, and parasites, account for almost two thirds of all known human infections. Some helminthoses that commonly infect canids and felids are typically soil-transmitted. This chapter focuses on two major groups of STHs that cause disease in humans: (a) the ascarids *Toxocara canis* and *T. cati*, associated with visceral and ocular larva migrans, and (b) the hookworms *Ancylostoma braziliense* and *Anc. caninum*, associated with cutaneous larva migrans. We review the current geographic distribution, laboratory diagnosis and clinical spectrum of these infections, examine the relative contribution of some risk factors for infection and disease, and discuss potential control measures for reducing the burden of disease in companion animals and humans.

A third soil-transmitted ascarid species that can cause human disease is *Baylisascaris procyonis*, commonly found in raccoons in North America. Human infections are characterized by severe neurological disease, leading to death or long-lasting sequelae (Watts et al., 2006). Another nematode species, *Gnathostoma spinigerum*, has occasionally been found in biopsy sample from patients with suspected VLM. More recently species of *Toxocara* including *T. malayensis*, a parasite of the domestic cat, and *T. lyncus*, which infects the caracal, have been identified, but their role in human disease remains unknown (Despommier, 2003). Soil-transmitted larval infection with other common canine and feline hookworms, such as *Anc. ceylanicum*, *Anc. tubaeforme* and *Uncinaria stenocephala*, can also cause occasional dermatological lesions in humans, and *Anc. ceylanicum* can readily develop in adults causing severe enteritis (Bowman et al., 2010). These zoonotic infections have significant public health implication in specific human populations, but are not reviewed in the present work.

2. *Toxocara canis* and *Toxocara cati*

The causal agents of human toxocariasis are the ascarid nematodes (roundworms) *T. canis* and *T. cati*, whose definitive hosts are dogs and cats, respectively. Although infection with these parasites has been described in their usual hosts for more than 200 years, only in the 1950s were they recognized as important human pathogens. When embryonated eggs are accidentally ingested, larvae hatch in the small intestine, penetrate the intestinal wall and migrate via the bloodstream to the liver, lungs, muscles, eye and the central nervous system. Although most infections are asymptomatic, two well-defined syndromes are classically recognized in human: visceral larva migrans (VLM), a systemic disease caused by larval migration through major organs, and ocular larva migrans (OLM), a disease limited to the eye and optic nerve. Less severe syndromes have been described mainly in children (covert toxocariasis) and in adults (common toxocariasis).

The genus *Toxocara* belongs to the order Ascaridoidea. The life cycle of *T. canis* is complex (Figure 1). Infections are acquired by oral ingestion of infective stages, but also by transplacental or transmammary routes, and may or may not include migration across the viscera of the definitive hosts. Female worms produce up to 200,000 eggs a day, which are shed in dog and cat feces and embryonate in the environment within 2-3 weeks, under ideal humidity and temperature (25-30°C) conditions. Akin to other ascarid eggs, fully embryonated ova contain third-stage larvae (L3). When dogs ingest embryonated eggs, L3 larvae hatch in the small intestine, penetrate the intestinal wall and are carried by the bloodstream to several tissues, particularly the liver and the lungs (entero-hepatic-pulmonar migration). The pulmonary L3 larvae undergo upward tracheal migration and swallowing, to return to the small intestine, where the final two molts take place. Although larvae will remain developmentally arrested in most adult dogs, they usually resume development in pregnant bitches and migrate across the placenta, infecting the foetus. After 4-5 weeks of infection, eggs shed by female worms are detectable in dogs feces, where the prepatent period is slightly longer (8 weeks) in *T. cati* infections of cats. Dogs and other canid species are also infected by transplacentary and transmammary migration of third-stage larvae.

Although *T. canis* is often regarded as the main, or sole, cause of human toxocariasis, the relative contribution of *T. cati* has possibly been underestimated because these two zoonotic species could not be reliably differentiated with the identification methods used in most studies (Fisher, 2003). Serosurvey data from Iceland, however, indicate that *T. canis* may be far more important than *T. cati* as a cause of toxocariasis (Overgaauw, 1997). Dogs have been banned from Iceland since the 1940s, as a measure to prevent human hydatid disease, but cats are allowed as pets. Nevertheless, all adult Icelanders exposed to cats (cat breeders and pet cats owners) tested to date have been seronegative for *Toxocara* (Woodruff et al., 1982).

The age of definitive hosts, particularly dogs, correlates negatively with the burden of infection with adult worms. Adult *T. canis* worms are most commonly observed in puppies up to three months of age. In dogs up to 5-6 months of age, tracheal migration of larvae usually results in the development of adult worms. At six months of age, the number of adult parasites in the intestine decreases drastically, putatively due to acquired immunity against migrating larvae, while L3 larvae typically undergo somatic migration and encapsulate in these hosts. In cats, usually more larvae undergo tracheal migration following ingestion of embryonated *T. cati* eggs in kittens than in older animals, but even adult animals may be susceptible to infection and disease.

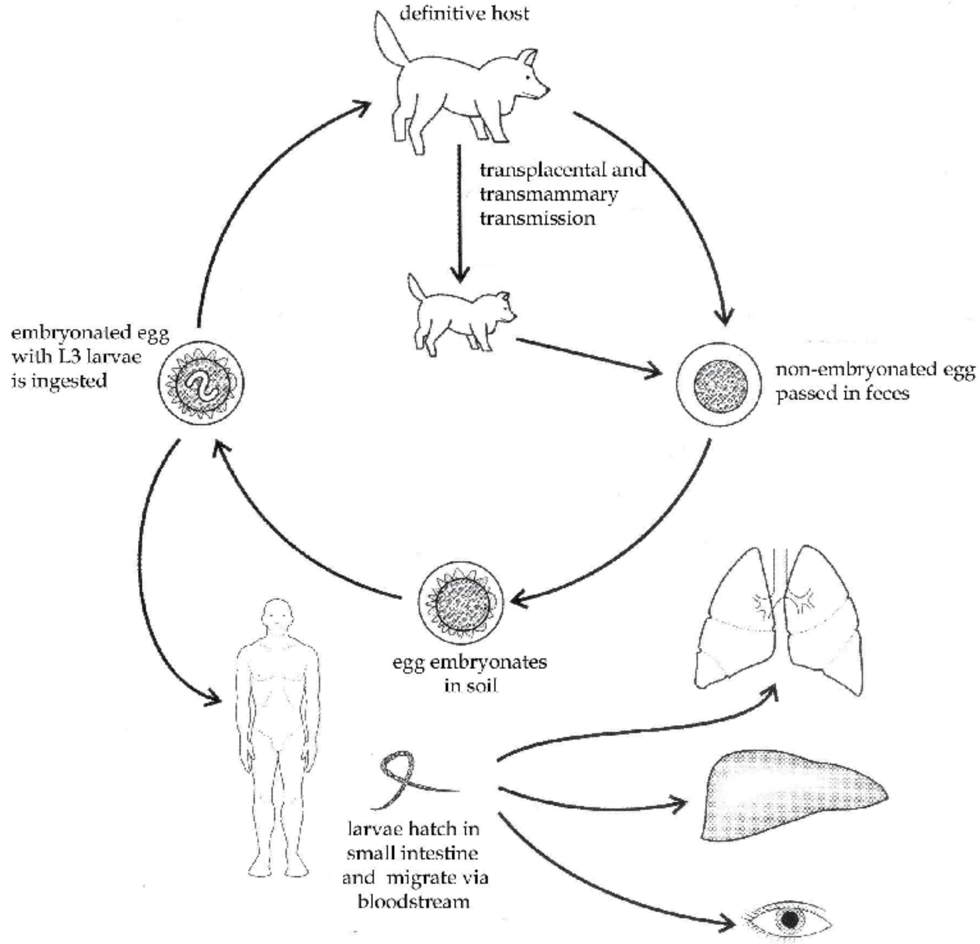

Fig. 1. Simplified life cycle of *Toxocara canis*. *T. cati* has a very similar life cycle, except that cats (instead of dogs) are the definitive hosts. Adapted from Despommier (2003).

T. canis larvae arrested in the tissues may be transmitted from bitches to offspring via the placenta (transplacental transmission). However, this phenomenon is not observed in *T. cati* infections of cats. During the last trimester of pregnancy of the bitch, tissue-arrested larvae become mobile due to hormonal influence. Larvae migrate to the lungs of the fetus. In the newborn puppy, the life cycle is complete when the larva migrates, via trachea, to the intestinal lumen, where the final molts take place. Adult worms can be found at two weeks of age, whereas large numbers of eggs can be detected in the feces after a minimum prepatent period of 16 days (Barriga, 1988, 1991).

Embryonated eggs present in environment may be ingested by a variety of accidental, paratenic hosts, such as rodents, sheep, pigs, cattle, birds, and humans. When such eggs from the soil or contaminated food are accidentally ingested by humans, L3 larvae hatch into small intestine, reach the lung and the heart and are carried to the systemic circulation.

3. *Ancylostoma caninum* and *Ancylostoma braziliense*

CLM is a relatively common clinical entity in humans caused by larval migration of zoonotic hookworms, mainly *A. braziliense* but also *A. caninum* and a few other species. Infective larvae penetrate the skin and migrate through the epidermis, but are usually confined to the dermis and do not develop into adult worms. Less common clinical manifestations are eosinophilic pneumonitis, localized myositis, folliculitis and erythema multiforme but eye involvement is rarely reported. Larval infection of humans with *Anc. ceylanicum* may occasionally give rise to adult worms that inhabit the small intestine and may cause abdominal discomfort and eosinophilic enteritis. The presence of immature *Anc. caninum* worms in the intestinal lumen of humans has rarely been reported (Bowman et al., 2010).

Infection of both definitive and paratenic hosts with these nematodes is most commonly acquired when third-stage hookworm larvae penetrate in their skin, although these infective larvae may also be ingested. In adult dogs infected with *Anc. caninum*, some larvae may undergo somatic migration and subsequently infect puppies by the transmammary route (Bowman et al., 2010; Soulsby, 1982). These larvae invade the skeletal muscle or gut wall and remain in an arrested state, becoming reactivated during the last two weeks of pregnancy (Barriga, 1988). Adult worms inhabit the small intestine of the definitive hosts (dogs for *Anc. caninum*, *Anc. braziliense*, *Anc. ceylanicum* and *U. stenocephala*; cats for *Anc. tubaeforme*, *Anc. braziliense*, *Anc. ceylanicum* and *U. stenocephala*) and may cause blood loss and anemia. Female worms shed eggs, typically two weeks after ingestion of larvae and about one month after skin penetration, which are passed in the host's feces. Once in the soil, first-stage larvae hatch and develop into infective third-stage larvae (Figure 2).

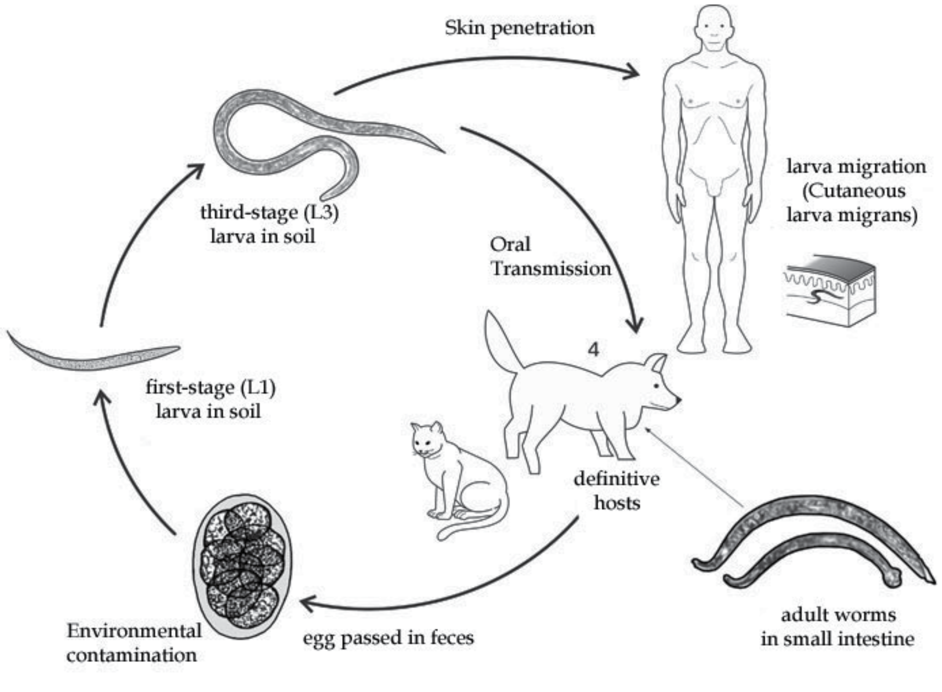

Fig. 2. Simplified life cycle of *Ancylostoma braziliense* and *Anc. caninum*. Adapted from CDC (2011).

4. Soil contamination with infective stages of zoonotic helminths

T. canis is transmitted to humans mainly by incidental ingestion of embryonated eggs present in the soil or soil-contaminated food (Acha & Szyfres, 2003). Since adult female worms produce large number of eggs and nearly all puppies are infected prior to birth, dog populations excrete a huge number of *Toxocara* eggs into the environment (Barriga, 1988).

Under favorable conditions (absence of direct sunlight exposure and appropriate temperature, humidity and oxygenation), particularly in tropical countries, *Toxocara* eggs can survive in the soil for several years. However, heavy environmental contamination has also been found in countries with temperate climate, such as Germany (Düwell, 1984) and Japan (Shimizu, 1993). Most (51-95%) eggs recovered from the soil of temperate countries were fully embryonated and, therefore, infective (Holland et al., 1991; Jarosz et al., 2010).

Humans can also be infected with *Toxocara* by ingestion of raw infected tissues of other paratenic hosts, such as cows, sheep or chicken, containing encapsulated larvae (Finsterer et al., 2010; Nakagura et al., 1989; Salem & Schantz, 1992). Food-borne transmission appears to be relatively common in East Asia (Akao & Ohta, 2007). Larval development progresses no further, but parasites can remain viable for up to seven years after infection (Smith et al., 2009). Although direct contact with infected puppies and kittens is not classically considered a risk factor for human toxocariasis, since the eggs shed by these animals must embryonate in the soil before becoming infective, these pet animals may carry embryonated eggs within their fur (Wolfe &Wright, 2003), in a small numbers (Overgaauw et al., 2009).

The species most commonly involved in human CLM is *Anc. braziliense*. Eggs shed within the feces of infected hosts hatch in the soil and develop into third-stage larvae in the environment. Human infection occurs through contact with contaminated soil of beaches, parks and schools (Bowman et al., 2010).

Eggs of *Toxocara* and zoonotic hookworm larva are found in soils worldwide, especially in public parks, playgrounds, sandpits, and beaches. Reports of soil contamination with infective stages of *Toxocara* and hookworm in public areas are available for several countries (Table 1).

Environmental and technical factors, such as soil type, pre-processing sieving, washing, and re-suspension of sediment, solution employed for washing and flotation, and the specific density of flotation solutions are all presumed to influence the recovery of ascarid eggs (Coelho et al., 2001; Nunes et al., 1994; Oge & Oge, 2000; Ruiz de Ybáñez et al., 2000; Santarém et al., 2009; Santarém et al., 2010).

Other variables, such as climatic conditions (temperature, rainfall, sunlight, etc.) or the amount of herbage and the presence of animals, number amongst other important factors contributing to soil contamination that may influence recovery of eggs.

The presence of dogs and cats may also play an important role on soil contamination by agents of larva migrans. Cassenote et al. (2011) observed that the number of dogs frequenting parks had an impact on soil contamination in public spaces.

The lack of standardisation of techniques as well as the wide range of factors influencing the process of egg recovery can lead to false-negative results and underestimation of the occurrence of contamination, hampering comparison of findings of different reports, and the assessment of their implications for public health (Coelho et al., 2001).

Fahrion et al. (2010) observed that the mean sizes of *T. cati* (62.3 by 72.7 μm) and *T. canis* (74.8 by 86.0 μm) eggs recovered from feces differed statistically. According to Fogt-Wyrwas et al. (2007), the differentiation of *Toxocara* spp. eggs from soil by ocular microscopy is extremely difficult due to the similarity in morphological characteristics of *T. canis* and *T. cati* eggs. As a consequence, studies have been carried out in an effort to provide molecular techniques for amplification of *Toxocara* spp. DNA that can be applied in routine examinations.

Continent /Country	Site	Frequency (%)	Reference
Africa Niger	Kaduna	9.0^{A}	Maikai et al. (2008)
Americas U.S.A.	Connecticut	14.4^{T}	Chorazy & Richardson (2005)
Argentina	Buenos Aires	13.2^{T}	Fonrouge et al. (2000)
Brazil	Fernandopólis	79,36^{T} 6,9^{A}	Cassenote et al. (2011)
	Itabuna	47.9^{A}	Campos Filho et al. (2008)
	Mirante do Paranapanema	76.9^{T}	Santarém et al. (2010)
	Praia Grande	45.9^{A}	Castro et al. (2005)
	Ribeirão Preto	20.5^{T}	Capuano & Rocha (2005)
	São Paulo	29.7^{T}	Muradian et al. (2005)
	Sorocaba	53.3^{T}	Coelho et al. (2001)
Chile	Santiago	66.7^{T}	Castillo et al. (2000)
Venezuela	Ciudad Bolívar	61.1^{A}	Devera et al. (2008)
Asia Japan	Tokushima	63.3^{T}	Shimizu (1993)
Thailand	Bangkok	5.71^{T}	Wiwanitkit & Waenlor (2004)
Turkey	Ankara	45.0^{T}	Avcioglu & Burgu (2008)
	Kirikkale	15.6^{T}	Aydenizöz-Ozkayhan (2006)
Europe Ireland	Dublin	15.0^{T}	O'Lorcain (1994)
Italy	March	33.6^{T}	Hábluetzel et al. (2003)

Table 1. Frequency (%) of soil contamination of public areas by *Toxocara* spp. eggs^{T} and *Ancylostoma* spp. eggs/larvae^{A} in different continents.

Zhu et al. (2001) constructed specific primers for *T. canis* and *T. cati* DNA amplification by the PCR technique, by extracting genomic material from adult worms from dogs and cats. Previously, Jacobs et al. (1997) obtained genomic material from adult worms or from embryonated eggs collected from the uteri of female worm, and devised a polymerase chain reaction-linked to restriction fragment length polymorphism (PCR-linked RFLP) targeting the second internal transcribed spacer (ITS-2) of ribosomal DNA (rDNA) for *Toxocara* spp. and other zoonotic ascaridoid identification.

Subsequently, further studies based on Jacobs et al. (1997) have been undertaken to detect and differentiate *Toxocara* spp. in soil (Borecka, 2004; Fogt-Wyrwas et al., 2007; Borecka & Gawor, 2008) and in fecal samples (Fahrion et al., 2010).

Fogt-Wyrwas et al. (2007) developed a technique based on a step PCR method for identification of *T. canis* and *T. cati* in soil samples. First, the authors recovered eggs using a flotation technique. Genetic analyses were then carried out after the crushing the eggs by pressing a cover slip on a microscope slide, to produce the embryonic material. Successful results were obtained only when a single or large numbers of eggs were recovered from 40 g soil samples. Both *T. canis* and *T. cati* genetic material were amplified. Borecka & Gawor (2008) verified that the use of proteinase K enabled amplification of genomic DNA from the soil without the need to isolate eggs using flotation or to inactivate PCR inhibitors present in the sample, thus making PCR easier and less laborious for routine use.

Another method developed to amplify DNA is the loop-mediated isothermal amplification (LAMP) assay. Based on a previous review, Parida et al. (2008) stated that LAMP is characterized by the use of six different primers. Amplification and detection of a gene can be completed in a single step, by incubating the mixture of samples, primers, DNA polymerase with strand displacement activity and substrates, at a constant temperature. The result is based on naked eye observations of a white precipitate (magnesium pyrophosphate). Thus, the method does not require expensive equipments, such as thermal cyclers or reagents.

5. Global prevalence of soil-transmitted zoonotic helminth infections in humans and associated risk factors

Human *Toxocara* infection has a cosmopolitan distribution, but reliable prevalence estimates are hard to derive from available serosurvey data, which comprises demographically diverse population samples that may not be representative of the general population of their respective countries, provinces or cities (Table 2).

Continent/ Country	Site(s)	No. of samples	Positive (%)	Reference
Africa La Réunion	90 districts	387^{C}	92.8	Magnaval et al. (1994)
Americas U.S.A.	Various areas	20,395^{AC}	13.9	Won et al. (2008)
Argentina	La Plata	156^{AC}	46.9	Radman et al. (2000)
	Resistencia	206^{C}	37.9	Alonso et al. (2000)
Brazil	Assis Brasil and Acrelândia	606^{C}	21.5	Ferreira et al. (2007)
	São Paulo city	399^{C}	38.8	Alderete et al. (2003)
	Campinas	138^{AC}	27.7	Anaruma et al. (2002)
	Granada	403^{AC}	26.8	Rubinsky-Elefant et al. (2008)
	Pres. Prudente	252^{C}	11.1	Santarém et al. (2011)
	Teodoro Sampaio (settlement)	79^{AC}	21.5	Prestes-Carneiro et al. (2008)
	São Paulo city	338^{C}	26.9	Muradian et al. (2005)
	Sorocaba	180^{C}	38.3	Coelho et al. (2004)
Peru	Lima	303^{AC}	20.5	Espinoza et al. (2010)
Asia Iran	Sari City	1,210^{C}	25.0	Sharif et al. (2010)
Taiwan	Districts in East	329^{C}	76.6	Fan et al. (2004a)
Europe Spain	Santiago de Compostela	463^{A}	28.6	Gonzalez-Quintela et al. (2006)

Table 3. Seroprevalences for human toxocariasis on different continents. Adapted from Rubinsky-Elefant et al. (2010). (Population studied: adult^{A}; children^{C}).

Toxocariasis tends to be more prevalent in tropical settings, compared to temperate regions, while rural populations are usually more exposed than urban populations in the same region (Rubinsky-Elefant et al., 2010). Reported seroprevalence rates in apparently healthy subjects range from 2.4% in Denmark (3247 subjects ≤ 40 years old; Stensvold et al., 2009) to 92.8% in La Réunion (387 subjects> 15 years old; Magnaval et al., 1994).

Although some risk factors for toxocariasis have been identified in human populations, results remain largely inconsistent. Male gender, for example, was suggested to be associated with both higher (Alonso et al., 2000; Kanafani et al., 2006; Roldán et al., 2009; Won et al., 2008) and lower (Abo-Shehada et al., 1992; Magnaval & Baixench, 1993) risk of infection, whereas several large studies showed no association between gender and risk (Chieffi et al., 1990; Rubinsky-Elefant et al., 2008. Young age (Fan et al., 2004b; Rubinsky-Elefant et al., 2008), low socioeconomic status (Campos Junior et al., 2003; Lynch et al., 1988a; Santarém et al., 2011; Won et al., 2008), low parental education (González-Quintella et al., 2006; Won et al., 2008), poor sanitation (Alderete et al., 2003; Magnaval et al., 1994) and playing in sandpits (Paludo et al., 2007) are additional factors contributing to *Toxocara* exposure.

Having a dog has been recognized as a risk factor in most (Chiodo et al., 2006; Fan et al., 2004b; González-Quintella et al., 2006; Jarosz et al., 2010; Won et al., 2008), but not all studies of human toxocariasis (Ajayi et al., 2000; Rubinsky-Elefant et al., 2008). Discrepancies are not altogether surprising, especially in tropical settings where dogs roam freely and spread eggs across large areas. As a result, infection may be acquired, especially in sandpits of children's playgrounds, regardless of the presence of pet dogs in the households. The contribution of cat ownership to *Toxocara* seropositivity has been less studied. Having cats as pets has been described, in two recent serosurveys in Brazil, as representing both a risk (Paludo et al., 2007) and a protective factor (Rubinsky-Elefant et al., 2008). A third survey in Poland (Jarosz et al., 2010), but not a large nationwide study in the United States (Won et al., 2008) found cat ownership to be a significant predictor of *Toxocara* seropositivity.

The antigen used in ELISA (Enzyme-linked immunosorbent assay) contains both species-specific epitopes and epitopes that are shared between *T. canis* and *T. cati* (Kennedy et al., 1987). If species-specific epitopes predominate, serology would preferentially diagnose exposure to *T. canis*. Nevertheless, if cross-reactive epitopes predominate and exposure to *T. cati* is frequent, ELISA would be unable to distinguish between exposure to *T. canis* and *T. cati* with both dog and cat ownership emerging as a risk factor for seropositivity.

Positive associations have been described between *Toxocara* seropositivity and current infection with other nematodes, such as whipworm (Cancrini et al., 1998) and hookworm (Rubinsky-Elefant et al., 2008). These results may reflect some degree of cross-reactivity of antibodies to TES with proteins excreted by other tissue- or lumen-dwelling nematodes. Although test sera in most laboratories are pre-incubated with an *Ascaris suum* extract to prevent cross-reactivity with this common human nematode (Elefant et al., 2006), other highly prevalent helminths may still elicit cross-reactive antibodies (Lynch et al., 1988b). Alternatively, *Toxocara* and other soil-transmitted helminths may co-infect the same host due to the similar ways of acquiring these infections.

Prevalence and geographic distribution of infections with zoonotic hookworms in humans and their definitive hosts remain relatively unknown (Bowman et al., 2010). As a rule, human CLM is more prevalent in children living in regions with warm and humid climates. *U. stenocephala* infects dogs and cats in the Americas, Europe, Asia and Oceania, while *Anc. ceylanicum* is commonly found in South and Southeast Asia, Australia, and most parts of South America. *Anc. braziliense* can be found from the southeastern coast of North America (but not on the Pacific Coast of United States and Mexico) down to South America, in African countries and Southeast Asia, but less frequently in Australia.

Human CLM, known or presumed to be caused by *Anc. braziliense,* has been reported in many tropical and subtropical regions, including North and South America, Southern Europe, India, and the Philippines. The distribution of human infection overlaps with the geographic range of *Anc. caninum*. CLM is the most common dermatologic condition that affects North American and European tourists returning from tropical countries. These imported cases are often reported after exposure to beaches in regions where *Anc. caninum* is commonly found in their definitive hosts.

6. Laboratory diagnosis, clinical spectrum and treatment of human toxocariasis

Because larvae do not develop into adult worms in humans, these paratenic hosts do not pass *Toxocara* eggs in their feces. As a consequence, fecal examination does not contribute to the laboratory diagnosis of human toxocariasis. Definitive diagnosis of current infection can only be obtained by histological examination of infected tissue, but biopsies are rarely obtained for diagnostic purposes. Less commonly, ultrasonography, computed tomography, and nuclear resonance imaging are also used to detect and localize lesions suggestive of granulomas (Magnaval et al., 2001; Watthanakulpanich, 2010).

Virtually all *Toxocara* infections in humans are diagnosed serologically. The standard test to diagnose human toxocariasis is the indirect ELISA with antigens excreted-secreted by *T. canis* (TES) L3 larvae (de Savigny, 1975, 1979). The TES-based ELISA for IgG antibodies has been reported to be 78% sensitive and 92% specific (Glickman et al., 1986), although putatively more specific recombinant antigens have been obtained for serology. Since cross-reactive antibodies elicited by exposure to other helminths may reduce the specificity of TES-based serology in tropical populations (Lynch et al., 1988b; Watthanakulpanich et al., 2008), serum samples are usually pre-incubated with antigens of related nematodes, to remove cross-reacting antibodies. Our test samples are routinely pre-incubated with an adult worm extract of *A. suum* (Elefant et al., 2006).

Positive ELISA results can be confirmed by Western blot (Magnaval et al., 1991), but this technique is more expensive and labour-intensive than ELISA. Recombinant *T. canis* antigens, which are species-specific, have been expressed and used in prototype ELISAs for detection of antibodies, with promising results (Yamasaki et al., 2000). Among the four human IgG subclasses, specific IgG2 antibodies to TES antigens yield the highest sensitivity in ELISA (Watthanakulpanich et al., 2008), while detection of IgG4 antibodies contributes to increased specificity (Noordin et al., 2005).

Immunoblotting (IB) techniques, based on TES antigens, have been applied to improve serodiagnosis, and for follow-ups after chemotherapy (Rubinsky-Elefant et al., 2011).

Antigen-capture ELISAs with monoclonal antibodies have been developed (Gillespie et al., 1993; Robertson et al., 1988) but poor specificity precludes their use in routine diagnosis. Polymerase chain reaction (PCR)-based methods for *Toxocara* identification in clinical and environmental samples have been described (Fogt-Wyrwas et al., 2007; Zhu et al., 2001), but are not widely available.

The clinical spectrum of toxocariasis in humans, ranging from asymptomatic infection to severe organ injury, is determined by parasite load, sites of larval migration, and the host's inflammatory response. Two severe clinical syndromes are classically recognized: VLM (systemic disease caused by larval migration through major organs) and OLM (disease limited to the eye and optic nerve). Half a century ago, Beaver and colleagues described

Toxocara larvae in eosinophilic granulomas in the liver of young children with extreme eosinophilia, hepatomegaly, respiratory symptoms, anemia and geophagia, and introduced the term VLM to describe this clinical syndrome (Beaver et al., 1952). Wilder found nematode larvae in eosinophilic granulomas of enucleated eyes of children with suspected retinoblastoma, providing the first description of the condition currently known as OLM (Wilder, 1950).

Classical VLM occurs typically in children aged 2-7 years, but infections in adults, at least some of which are acquired by ingesting raw organs of paratenic hosts, are relatively frequent in East Asia (Akao & Ohta, 2007). The full-blown VLM syndrome usually includes fever, lower respiratory symptoms such as cough, dyspnea and bronchospasm associated with larval migration, hepatomegaly, abdominal pain and decreased appetite. Laboratory findings include hypergammaglobulinemia, increased isohemagglutinin titres to A and B blood group antigens, anaemia and leukocytosis with marked eosinophilia (Jacob et al., 1994). As a rule, seropositive subjects in population-based surveys are asymptomatic or have rather nonspecific and mild symptoms. A case-control study in Ireland led to the description of a new clinical entity in seropositive children, called "covert toxocariasis", comprising mainly fever, headache, behavioural and sleep disturbances, cough, anorexia, abdominal pain, hepatomegaly, nausea and vomiting (Taylor et al., 1987). Another case-control study, in French adults, led to the definition of "common toxocariasis", a syndrome comprising chronic dyspnea and weakness, cutaneous rash and pruritus, as well as abdominal pain (Glickman et al., 1987).

The liver is the most commonly affected visceral organ. Typical hepatic granulomas have multinucleated giant cells and epithelioid cells surrounding necrotic debris or amorphous eosinophilic material. Eosinophils and mononuclear cells are often seen in the outer layers of the granulomas (Musso et al., 2007). On computed tomography, hepatic lesions are typically ill-defined, low-attenuating nodules (Cameron et al., 1997) that have sometimes been confounded with metastatic cancer (Ota et al., 2009).

The cutaneous manifestations of human toxocariasis have been recently reviewed (Gavignet et al., 2008) and include chronic prurigo, pruritus and urticaria, eczema, exanthema (Bernardeschi et al., 2011), and vasculitis.

Central nervous system involvement in toxocariasis comprises eosinophilic meningitis and encephalitis (Moreira-Silva et al., 2004), myelitis (Lee et al., 2009), cerebral vasculitis (Helbok et al., 2007) and optic neuritis, while manifestations of peripheral nervous system involvement include radiculitis (Moreira-Silva et al., 2004) and cranial nerve palsy (Finsterer & Auer, 2007). Central nervous system involvement in VLM has been associated with epilepsy (Woodruff et al., 1966), behavioral changes and cognitive deficits. *Toxocara* may represent a co-factor in idiopathic seizures (Critchley et al., 1982), and especially in partial epilepsy (Nicoletti et al., 2007). The presence of granulomas in the brain has been suggested to elicit focal seizures (Critchley et al., 1982). Research to verify increased risk of cognitive deficits in infected children has remained inconclusive (Jarosz et al., 2010).

There are very few controlled trials on anthelmintic drugs for VLM in the literature. Since parasitological cure in patients cannot be assessed, the end-point of published trials is a decrease in the severity of clinical signs and symptoms. A dose of 500 mg of albendazole twice a day for 5 days is currently recommended. Albendazole seems to be superior to thiabendazole (50 mg/kg of body weight daily for 3-7 days) (Stürchler et al., 1989). Diethylcarbamazine (3-4 mg/kg of body weight daily for 21 days, starting at 25 mg/day and increasing the dose progressively) is also effective (Magnaval, 1995). Most human infections with *Toxocara*, however, cause much less severe systemic manifestations, if any, and treatment is not required.

Eosinophilia and elevated levels of IgE are commonly found in toxocariasis, as well as high titres of *Toxocara* antibodies. Covert and common toxocariasis are likely to represent slight variations in the clinical spectrum of mild infections in children and adults, respectively.

Although wheezing is a common presenting feature of VLM, whether or not *Toxocara* infection predisposes to asthma remains uncertain. Some epidemiological studies have shown a positive association between wheezing or asthma and *Toxocara* seropositivity (Desowitz et al., 1981; Ferreira et al., 2007; González-Quintella et al., 2006), while others failed to detect such an effect (Fernando et al., 2009; Sharghi et al., 2001). Asthma symptoms can result from larval migration through the lungs, but a role has also been proposed for parasite-induced atopy (Cooper, 2009).

Compared with systemic disease, ocular toxocariasis or OLM usually affects older children, with an average age at onset of 7.5 years (range, 2-50 years) (Taylor, 2001). About 80% of cases are diagnosed in patients younger than 16 years of age (Brown, 1970). Males tend to be more frequently affected than females (Brown, 1970; Taylor, 2001). The clinical condition currently known as OLM or ocular toxocariasis was first described by Wilder (1950), who found nematode larvae or their residual hyaline capsules during the histological analysis of 24 eyes that had been enucleated from children with suspected retinoblastoma.

The clinical presentation of OLM depends on the primary anatomic site involved and the immune response of the host. A single eye is affected in most patients (Taylor, 2001). The most common symptoms are strabismus, unilateral decreased vision and leukocoria (white eye). Peripheral, posterior pole retinal granuloma and endophthalmitis are the usual presentations on the eye exam.

The presence of a vitreous band, or a membrane extending between the posterior pole and high-reflective peripheral mass, detected by ocular ultrasound, may help in the diagnosis when the ocular medium is opaque (Figure 3).

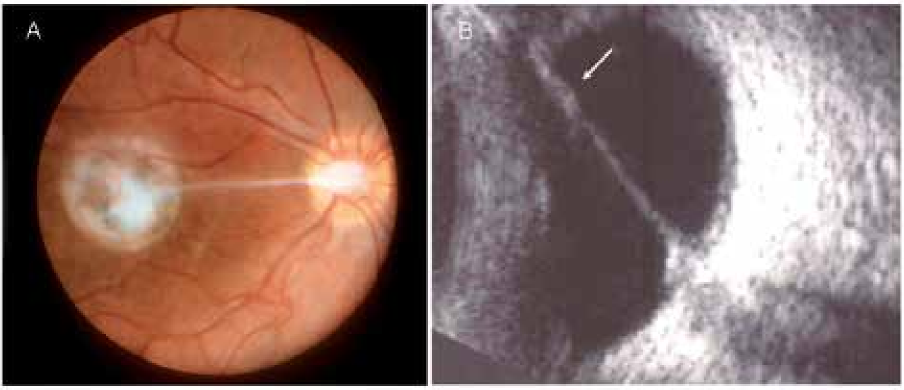

Fig. 3. Clinical presentation of ocular toxocariasis. A. Peripheral retinal and vitreous lesion, a localized mass of whitish tissue involving the retina and peripheral vitreous with a fibro-cellular band running from the periphery toward the optic nerve or posterior retina. B. Ocular ultrasound showing a vitreous band or membrane (arrow) extending between the posterior pole and high-reflective peripheral mass. (Image Courtesy: Ophthalmology Clinic of the University of São Paulo - Medical School General Hospital, HC-FMUSP).

Other uncommon clinical presentations of OLM include optic disc inflammation (papillitis or neuroretinitis), motile intraocular nematode (retina, vitreous body and anterior chamber), keratitis and cataract.

The diagnosis of OLM is usually suggested by the presence of the clinical findings mentioned above, but the detection of specific antibodies is required. However, serum antibodies can often be undetectable (Sharkey & McKay, 1993), possibly due to the relatively low parasite load in these infections (Schantz, 1989). Even low ELISA serum titres may be of diagnostic value in OLM, but there is no consensus on the cut-off titres for diagnosis. Specific antibodies can be also detected in the aqueous humor (AH). The intraocular production of antibodies to *Toxocara* can be assessed by comparing serum and AH samples obtained simultaneously from the same patients and calculating the Goldmann-Witmer (GW) coefficient as: ([levels of specific IgG in AH/levels of specific IgG in serum]/[total IgG in AH/total IgG in serum]). A GW coefficient > 3 indicates intraocular production of specific antibodies (De Visser et al., 2008).

Sight-threatening ocular inflammation secondary to OLM requires aggressive anti-inflammatory therapy, combined with albendazole (800 mg for adults and 400 mg for children daily) over a 2-4 week period. Oral steroids (prednisone 0.5 mg/kg/day) are used to reduce the inflammatory response induced by larvae. Surgical treatment may be required for retinal detachment or intravitreal fibrovascular membrane proliferation.

7. Cutaneous larva migrans

CLM is a clinical entity caused in humans by larval migration of zoonotic hookworms, mainly *Anc. braziliense* but also *Anc. caninum*. Less common clinical manifestations are eosinophilic pneumonitis, localized myositis, folliculitis and erythema multiforme but eye involvement is rarely reported. Human infection occurs when infective L3 larvae penetrate the skin. The infection site may or may not present an erythematous popular or vesicular rash.

The larvae do not undergo further molts in the human host, but as they migrate across in the skin, at a rate of 2.7 mm per day, they leave an erythematous, serpiginous track. The cutaneous lesions can last several weeks and may be severely pruritic, but eventually resolved spontaneously. Secondary bacterial infection may result from scratching.

The most commonly affected sites are those in close contact with the soil (Araújo et al., 2000). A recent case series of CLM in Brazil, for example, showed that cutaneous lesions are more frequently observed on the feet (73.3%), buttocks (14.7%), genital and inguinal areas (8.0%), legs (2.7%), and hands (1.3%) (Jackson et al., 2006). Other sites, such as the face (Bouchad et al., 2000) and the scalp (Guimarães et al., 1999), are rarely affected. Clinical diagnosis is reached based on the typical skin lesions, while biopsies have little diagnostic value, showing an eosinophilic inflammatory infiltrate. Chemotherapy is seldom needed, since larvae die out within a matter of weeks if left untreated, but oral albendazol (400-800 mg/day for 3-5 days), oral ivermectin (200 µg/kg, single dose) or topical thiabendazole (10% aqueous suspension four times a day) may be used.

Larval infection of humans with *Anc. ceylanicum* may occasionally give rise to adult worms that inhabit the small intestine and can cause eosinophilic enteritis (Bowman et al., 2010). In addition to cutaneous lesions, *Anc. caninum* has also been reported to cause eosinophilic enteritis and may be a cause of diffuse unilateral subacute neuroretinitis in humans (Sabrosa & de Souza, 2001).

8. Prevention and control of zoonotic soil-transmitted helminth infections in humans and companion animals

Contaminated soil is the most important route of transmission of zoonotic helminths to humans. Environmental contamination is particularly relevant when public areas (parks, playgrounds, beaches) are affected. Effective preventive measures include covering sandboxes in public parks and playgrounds when not in use, allowing no dogs and cats on bathing beaches, and controlling stray dog and cat populations.

In southeastern Brazil, Santarém et al. (2004) reported a significant decrease in the incidence of LMC after replacement of soil in sandboxes and enclosure of playground areas with fences. Also in southern Brazil, Cassenote et al. (2011) observed that the frequency of geo-helminthes in fenced parks (11.1%) was significantly lower than that verified in non-fenced off areas (45.3). Similarly, Avcioglu & Balkaya (2011) observed in Turkey that fenced parks were free of *Toxocara* eggs, while 64.3% of open areas were contaminated with eggs.

Periodic prophylactic deworming of companion animals and educational measures aimed at pet owners are also critical for controlling infections by soil-transmitted helminths (Stull et al., 2007). A strategic program for decreasing soil contamination with zoonotic helminths should include elimination of intestinal parasites from puppies and kittens. Since puppies and kittens harbor adult *Toxocara* and hookworm due to infections via placenta and/or milk, treatment must target newborn animals, before eggs are first shed in the feces.

The WHO (2011), based on considerations by Barriga (1988, 1991), currently recommends treatment for puppies at two weeks of age to eliminate larvae acquired through transmammary or transplacentary transmission. Treatment is repeated at 4, 6 and 8 weeks. For kittens, treatment must be done at third, fifth, seventh and ninth weeks of life to eliminate the larva passed through milk. A single dose of anthelmintic for queens, 10 days after delivery, is also recommended (Barriga, 1991). Laboratory confirmation of infection, with stool examination using concentration methods (most often based on flotation procedures), is normally required prior to treatment of animals older than six months of age, to prevent uncontrolled use of anthelminthic drugs and the emergence of resistant parasites.

Veterinarians are thought to be on the 'front line' of prevention of pet-associated zoonotic parasitic infections (Smith et al., 2009). However, recent surveys have revealed that veterinarians often misinterpret and misuse the available protocols for deworming newborn pets. In Canada, 80-90% of the protocols recommended for puppies and kittens were inappropriate (Stull et al., 2007), and in the USA only 16% of the veterinarians interviewed knew how to deworm puppies (Harvey et al., 1991). In addition, veterinarians' perception concerning small animal-derived zoonoses should be improved, with emphasis on their role in disseminating information about these diseases to their clients (Stull et al., 2007).

Human infections with canine and feline helminths ranks among the most common zoonotic infections worldwide, yet remain relatively unknown to the public and pet owners. Katagiri & Oliveira-Siqueira (2008), in São Paulo, Brazil, observed a low level of risk perception of zoonotic infection by dog owners in Brazil. Pet owners should know how to prevent environmental contamination and to reduce the risk of human infection with zoonotic helminths. This requires a clear understanding of zoonoses acquired from small animals, of the need for appropriate deworming strategies for pets, and the need for removing feces from the environment where their dogs evacuate.

The public must also be informed about the risks of exposing children to public parks and beaches frequented by animals and of eating soil or biting nails, as well as about the benefits of washing hands after handling fecal material or playing with pets.

The American Veterinary Medical Association (AVMA, 2008) considers that the convergence of people, animals, and our environment has created a new dynamic in which the health of each group is inextricably interconnected. The Association proposed a holistic, collaborative approach aimed at improving animal and human health globally through collaboration among all the health sciences, especially between the veterinary and human medical professions to address critical needs.

9. Conclusion

Based on the findings in this review, it can be asserted that the lack of standardisation of techniques coupled with the host of factors influencing the process of egg/larvae recovery can lead to false-negative results and underestimation of the occurrence of soil contamination. Thus, the development of new methods is necessary to provide more reliable data under field conditions. Molecular analyses, based on amplification of genetic material extracted from eggs/larvae present in soil, are promising techniques both for identifying and characterizing of helminths present in soil.

It was also observed that soil contamination in public areas can be reduced by adopting a number of measures including: restriction of uncontrolled dogs and cats, cleaning up dog feces from soil and pavements by their owners, preventing access of dogs and cats to public spaces (especially children's playgrounds) and by use of strategic anthelmintic treatment of dogs and cats with emphasis on puppies, kittens, nursing bitches and queens.

Programs designed through collaborative efforts of both human and veterinary doctors/researchers are essential to create fresh tools for diagnosis and new strategies for controlling the transmission of soil-transmitted helminthic zoonoses to humans, until new technologies become available.

According to the WHO (2011), one of the main strategies for controlling zoonotic diseases is to promote advocacy so as to emphasize their burden on society and create demand at all levels of society to control them. The American Veterinary Medical Association (AVMA, 2008) has considered that the convergence of people, animals, and our environment has created a new dynamic in which the health of each group is inextricably interconnected. The Association has proposed a holistic, collaborative approach aimed at improving animal and human health globally through collaboration among all the health sciences, particularly between the veterinary and human medical professions to address critical needs.

Many diseases are considered neglected zoonotic diseases, including soil-transmitted helminths. Thus, efforts to design public educational programs raising awareness of agents of larva migrans are fundamental to prevent the burden of diseases in companion animals and humans. Further, improvements in diagnostic testing and expansion of epidemiologic surveillance should be promoted in parallel with control and prevention efforts.

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4

Reflectance Spectroscopy as a Tool for Monitoring Contaminated Soils

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1. Introduction

Soil reflectance spectroscopy is a well-known technique to assess soil properties rapidly and quantitatively in both point (spectroscopy) and spatial (imaging spectroscopy (IS)) domains. The quantitative approach has been developed in the past two decades by many researchers, with much literature on the topic. Basically, this approach was adopted from other disciplines (e.g. food, textiles), whereas the mapping approach was incorporated with spectral-based remote sensing means. Soil contamination is an ever-growing concern, and a reliable and environmentally friendly method is needed to rapidly detect and monitor soil contaminants at any stage for the diagnosis of suspected contaminated areas, as well as for controlling rehabilitation processes. Conventional methods for investigating soil contamination based on point sampling and chemical analysis are time-consuming, costly and sometimes incorporate the use of environmentally damaging chemicals. Soil reflectance spectroscopy is a promising tool for the efficient detection and monitoring of soil contaminants. Using reflectance spectroscopy measurements, several direct and indirect soil properties, as well as soil contamination characteristics, can be extracted. Due to the vast amount of data in soil spectroscopy, be it point or image, data-modeling processes are required. This chapter reviews soil spectroscopy theory and its quantitative capabilities, as well as data-modeling methods for soil spectra that are related to soil contamination. Discussed contaminants include petroleum hydrocarbons, heavy metals such as Ni, Cr, Cu, Cd, Hg, Pb, Zn and As, acid mine drainage and pesticides. Limitations, obstacles and problems in recording soil spectra in the field and in the laboratory are discussed, along with IS technology. The future potential of spectral technology and IS missions used for soil contamination monitoring is presented, based on ongoing worldwide initiatives. This chapter also provides some notes on how to merge this technology with other systems, such as a penetrating spectral device for underground measurements utilizing the catheterization approach.

1.1 Energy matter interactions

Most, if not all matter interacts with electromagnetic radiation in one way or another. Electromagnetic radiation that is radiated onto any kind of material may be reflected, absorbed or transmitted. For most materials, specific light interactions of a certain wavelength can be observed in the same range as solar radiation (Hunt 1980). For that reason, this chapter focuses on this radiation range (VNIR-SWIR, 400 to 2,500 nm). This range is divided into two sub-ranges: VNIR (400 to 1,100 nm) and SWIR (1,100 to 2,500 nm). Interactions between light and matter are wavelength-dependent. While electromagnetic radiation radiated onto a specific material may be reflected at one wavelength, it might be absorbed at another. This means that the reflected radiation of a material is a function of wavelength, surface material absorption, emissivity and reflectance characteristics. Therefore, energy-matter interactions in the VNIR, SWIR and IR (2,500 to 12,000 nm) wavelength regions can be used for qualitative and quantitative chemical analysis of a material.

1.2 Spectroscopy

VNIR-SWIR and IR spectroscopy has been used for qualitative and quantitative analysis for several decades (Taylor et al. 1970; Stark et al. 1986). Both are based on characteristic vibrations of chemical bonds in molecules, as well as electronic processes (the latter affects only radiation at 400 to 1,000 nm) (Ben-Dor et al. 1999). In general, incident IR is absorbed by the chemical-bond vibrations in the molecules (3N-6 fundamental possibilities where N is the number of atoms in a given molecule). The combination modes and overtones of the fundamental vibrations in the IR region are observed in the VNIR-SWIR region, and can be detected if the incident radiation in this latter region interacts with the matter in question. Whereas using the IR region in the laboratory requires sample preparation and caution during the measurements (e.g. not to exceed the saturation point), the VNIR-SWIR region has the advantage of allowing direct measurement of the material without complicated pretreatment and reagents. Another advantage of VNIR-SWIR over IR spectroscopy is that it is a non-destructive and non-contact method: it allows real-time measurements based on a simple illumination source (e.g. sun, tungsten halogen bulb). It is therefore suitable for on-line, *in-situ* monitoring and analysis of many types of compounds, mixtures and materials. In contrast, IR spectroscopy is a laboratory analysis method that requires considerable sample preparation. Its advantage over VNIR-SWIR lies in the fact that all chemical bonds within the molecule are active in this region, whereas in the VNIR-SWIR region only some of the bonds are active (e.g. OH-, NH-). Another benefit of IR is that it can be interpreted directly based on known chemical bonds, while in the VNIR-SWIR region, the spectra must be studied in advance to evaluate which wavelengths will best represent a given property (this is due to the possible overlap between some combination modes and overtones in the studied material). As a result, complex chemometric processes are used for the interpretation of VNIR-SWIR spectra into a quantitative model, in a process termed NIRA or NIRS (near infrared analysis). In this chapter, we will focus on hyperspectral data (continuous spectral data, amounting to hundreds of spectral bands), in the field of soil-contamination monitoring by reflectance spectroscopy and NIRS.

1.3 Soil spectroscopy

Today, the NIRS technique is well-known and utilized in many disciplines, such as food science, pharmacology, textile, tobacco, and oil industries, among others. Since the mid-1960s, when Bower and Hanks showed that soil moisture is highly correlated with soil spectra (Bowers & Hanks 1965), soil has captured the attention of many researchers who have realized that despite its complexity, soil spectroscopy can provide remarkable amounts of quantitative information. Stoner et al. (1980) were the first scientists to systematically gather soil spectral information and publish it in the form of a soil spectral atlas. Their soil spectral library very soon became a classic tool that soil scientists came to rely on. Later, when laboratory and portable field spectrometers were introduced into the market (around 1993), more scientists realized the potential of soil spectroscopy, and consequently more spectral libraries were assembled and new quantitative, chemometric applications, such as NIRA, were developed and implemented for various soil materials. A summary of soil reflectance theory and its applications can be found in various publications (Irons et al. 1989; Ben-Dor et al. 1999; Ben-Dor 2002). A study by Brown et al. (2006) showed that NIRA can work successfully under a generic global view rather than relying on a specific soil population. An extensive study with over 1,100 soil samples, using several data-mining algorithms to model and interpret diffuse reflectance spectra of soils and predict a number of soil attributes was recently performed by Rossel & Behrens (2010).

1.4 Imaging spectroscopy and remote sensing

Imaging spectroscopy (IS), or hyperspectral remote sensing, is an advanced tool that generates data of high spectral resolution, with the aim of providing near-laboratory-quality reflectance (or emittance) for each single picture element (pixel) from a far distance (Vane et al. 1984). This information enables the identification of objects based on the spectral absorption features of chromophores and has been found very useful in many terrestrial and marine applications (Clark & Roush 1984; Dekker et al. 2001). IS brings a new dimension to the field of remote sensing by expanding the envelope of point spectrometry to the spatial domain. It provides a tangible perspective by adding spatial detail to spectral information, thereby enhancing the thematic application of spectral recognition algorithms. This capability exists for both far and close distances, such as data acquired by satellites or by microscopic sensors, respectively. Whereas the former is used for mapping the earth from space, the latter is used for mapping micro targets, such as microorganisms and cell bodies, to account for their biochemical processes in a spatial domain (Soenksen et al. 1996; Levenson & Farkas 1997). It is interesting to note, however, that although soil scientists have recognized the potential of reflectance spectroscopy and in fact termed it a novel technology, in many ways, the use of IS for soil applications remains undeveloped and is seldom reported. Though the IS approach is a cost-effective method, its adoption is limited because the data is difficult to process, only a few sensors are operated worldwide, and it has not yet been recognized by many end users. Hence, the journey from point spectroscopy to a cognitive (imaging) spectral view of soils has not yet been completed, although there is no doubt that it may open up new frontiers in the field of soil science. Thus far, only exclusive and select groups around the world have been able to use IS for soil applications. Nevertheless, over the past 10 years, these groups have demonstrated remarkable achievements and have documented its significant capability. For further information refer to the chapter on "Optical remote sensing techniques for soil contamination monitoring and vulnerability assessment" in this book.

1.5 Acquiring reflectance information from soil

To acquire reflectance information from the VNIR-SWIR region, several sensors, methods, protocols and platforms are used. Basically, the VNIR-SWIR sensors consist of an apparatus (with filters, grating, prisms, interferometer, etc.) that splits the measured radiation into individual wavelengths (or regions), several detectors (mostly CCDs) which are sensitive to different regions), and various other optional optical components (lenses, collimators, beam-splitters, integrating spheres, optical fibers, etc.). The radiation source is usually a tungsten halogen light bulb when recording spectra under laboratory conditions, and the sun when recording spectra in the field. These sensors are generally periodically calibrated to provide the radiance values but in practice, the reflectance is calculated against a white reference target (Spectralon) that is assumed to reflect 100% of the radiation in the sample geometric configuration. There are point and image sensors. The point sensor records a single spectrum of the target (with a line-array detector) while averaging the mixed information of all components within the sensor's field of view. The size of the single pixel being measured depends upon the optics and the distance from the target. The image sensor provides data similar to the point sensor except that an area-array detector is used that enables the acquisition of spectral-cube information constructed from spatial and spectral views of the area (target) in question. Image sensors usually have lower spectral resolution as well as lower signal-to-noise ratio, as the integration time over the targets is less than in the point spectrometer measurements. Laboratory sensors are more accurate, with higher spectral resolution and a better signal-to-noise ratio: the integration time is long and the geometric scheme is constant. They usually include a sample holder and a stable light source. Field sensors are generally battery-operated and are less accurate than laboratory sensors. They rely on the sun's radiation and the geometric measurement is not fixed. Nonetheless, they provide flexibility and *in-situ* measurement capabilities that cannot be achieved under laboratory conditions. The field sensors are a valuable tool for recording reference spectra for space- or airborne remote sensing imagery which will be later used for calibration, validation and quality control (Brook & Ben-Dor 2011). Image sensors can be operated from both ground and air (space) domains. They can be integrated with the aircraft's GPS/INS system and produce multi or hyperspectral data cubes that can be georeferenced; thus each pixel in the image corresponds to a single area unit on the ground and is represented by a spectrum. The signal-to-noise ratios of air- and spaceborne sensors are lower than those operated on the ground and are governed by many factors (e.g. pixel size, integration time, frame rate, electronic noise, IFOV and atmosphere attenuation). A comprehensive review of image-based sensors for soil application can be found in (Ben-Dor et al. 2008).

2. Quantitative applications of spectroscopic data

Spectroscopic data are multivariate in nature. There are two approaches: 1) the chromophore absorption band in question is known and under saturation (supervised), and 2) the chromophore absorption band is unknown and is affected by several factors (unsupervised). Whereas the first approach is valid for the IR region by using selected wavebands and pretreatment to prevent saturation, the second represents the case of reflectance spectroscopy across the VNIR-SWIR region. For that purpose, multivariate statistical techniques (also called chemometrics) are required to extract the information about the quality attributes that is hidden within the spectral information. Essentially, this involves regression techniques coupled with spectral preprocessing.

2.1 Preprocessing

Spectral preprocessing techniques are used to remove any irrelevant information which cannot be handled properly by the modeling techniques. The preprocessing techniques include averaging, centering, smoothing, standardization, normalization and transformations, among others. Some of the most common preprocessing techniques are presented here. For a full review of preprocessing methods, please refer to Nicolaï et al. (2007).

2.1.1 Multiplicative scatter correction

Multiplicative scatter correction (MSC) is one of the most commonly used normalization techniques. In MSC, the light scattering is estimated for each sample relative to an ideal sample obtained by averaging the complete wavelength range of the data set. Each spectrum is then corrected such that all samples appear to have the same scatter level as the reference spectrum. For a further description of this method, see (Isaksson & Naes 1988; Naes et al. 1990; Vohland et al. 2009).

2.1.2 Absorbance

Spectroscopic measurements performed in transmission mode can be quantified using Beer's Law (concentration $\simeq \log(1/T)$ where T is the transmitted radiation). Accordingly, reflectance measurements are frequently converted to $\log(1/R)$ values, which are then used in a manner similar to optical density readings (Nicolaï et al. 2007).

2.1.3 Kubelka-Munck

The Kubelka-Munck transformation, $(1-R^2)/2R$, is similar to absorbance but accounts for scattering (Nicolaï et al. 2007).

2.1.4 Continuum removal

The continuum is the background absorption on which other absorption features are superimposed. The spectrum is divided by a convex hull that is fitted over it (Clark & Roush 1984; Clark & others 1999).

2.1.5 Derivatives

Derivation is often used to remove baseline shifts and superposed peaks. Second-derivative spectra can correct for both additive and multiplicative effects (like MSC). They are usually calculated according to the Savitzky-Golay algorithm (Naes et al. 2002). The parameters of the algorithm (interval width, polynomial order) should be carefully selected to avoid amplification of spectral noise (Nicolaï et al. 2007).

2.1.6 Spectral mixture analysis

Spectral mixture analysis (SMA) is a widely used method to determine the sub-pixel materials that fundamentally contribute to the spectral signal of mixed pixels. This is of particular importance for obtaining quantitative estimates of distinct materials, a typical application of remote sensing hyperspectral data. SMA aims to decompose the measured reflectance spectrum of each pixel into the proportional spectral contribution of so-called endmembers (EMs). In recent years, many authors have proposed and used a more complex model, in which both the number and the set of EMs vary dynamically on a per-pixel basis; this has become known as multiple EM SMA (MESMA). The idea consists of restricting the large set of possible EMs to a small set of better suited EMs, which can be different for each pixel, thereby allowing an accurate decomposition using a virtually unlimited number of

EMs. An improved strategy, called variable MESMA (VMESMA), allows segmentation of the image to increase flexibility and accuracy (Kemper & Sommer 2003).

2.2 Modeling

Modeling refers to relating a set of spectral parameters that are derived from the spectral information (before or after the aforementioned preprocessing treatment), to the real chemistry of the material in question. This is done by using a set of well-known samples as a training group. The data are divided into three groups: training, validation and test. The relationship between the chemistry and the spectroscopy data is found via the training group and simultaneously cross-validated by the validation group. Finally, the model is applied to the test group, independent of the training and validation process. Multivariate regression techniques are modeling methods that search for the relationship between two matrices: the spectral data matrix that can be very complex due to large amounts of data (X variables, the independent data), and a specific chemical reference value data matrix (Y variables, the dependent data). The common multivariate regression techniques are presented herein. For an in depth reference, please read further at (Esbensen et al. 2002; Nicolaï et al. 2007).

2.2.1 Multiple linear regression

Multiple linear regression (MLR) is a classical method that creates a linear combination of the spectral values at every single wavelength to correlate as closely as possible to the dependent reference values. The regression coefficients are estimated by minimizing the error between predicted and observed response values in a least squares sense. MLR models typically do not perform well with spectral data because spectral data usually exhibit high co-linearity, noise and more variables, i.e. more spectral bands, than measured samples (Esbensen et al. 2002; Nicolaï et al. 2007).

2.2.2 Principal component regression

Principal component regression (PCR) is a combination of principle component analysis (PCA) and MLR. The independent data matrix (spectral data) is transformed by PCA, and the first few principal components (PCs), which represent most of the independent data variance, are used as inputs for the MLR model instead of the original spectral data. The advantage over standard MLR is that PCs are uncorrelated, and the noise is filtered. The first few PCs are usually sufficient for a robust model and over-fitting issues can be eliminated. Although PCR consists of the two most studied multivariate methods (PCA and MLR), the major criticism against it is that the PCs (several first PCs) selected for the MLR input are not necessarily the best predictors for the reference data. There is no guarantee that the first PCs will include the spectral data related to the specific dependent variable that needs to be modeled (Esbensen et al. 2002; Nicolaï et al. 2007).

2.2.3 Partial least squares regression

Introduced in 1983 by Wold et al., partial least squares regression (PLS) is similar to PCR, but in PLS the PCs are constructed such that they include the chemical reference (Y variables, dependent data) in the calculation process. This technique orders the PCs according to their relevance for predicting the dependent variables, rather than to their description of the most variance of the spectral data. This method excels when the dependent data (X variables) express common information, as usually happens in spectral data. The required number of PCs is typically smaller than that in a PCR calibration model for similar model performance (Wold et al. 1983; Esbensen et al. 2002; Nicolaï et al. 2007).

2.2.4 Artificial neural networks

Artificial neural networks (ANNs) are based on their ability to "learn" during a training process where they are presented with inputs and a set of expected outputs. The basic structure of an ANN consists of three layers of "nodes" or "neurons": an input layer (i.e. spectral data or PCs), a hidden layer (which can consist of one of more nodes), and an output layer (which combines the outputs of the hidden layer into a single output). The node is a fundamental processing unit; each node has a series of weighted inputs, either from an external source or the output from other nodes. The inputs to the node are analogous to synapses, and the weights correspond to the strength of the synaptic connection. The sum of the weighted inputs is transformed with linear or nonlinear transfer functions, a popular nonlinear transformation function being the sigmoid function. The learning (or training) is an iterative process in which the resultant output is compared to the provided expected output, and an algorithm adjusts the weights accordingly. This method was first tested in the field of spectroscopy on simulated data (Long et al. 1990). It was proven to provide the best results in modeling soil constituents in a recent large-scale study that included over 1,100 soil samples (Rossel & Behrens 2010) .

3. Heavy metals

3.1 Introduction

Heavy metals are released into the environment—the air (e.g. during combustion, extraction and processing), surface water (via runoff and release from storage and transport) and soil (and hence into the groundwater and crops). Although the adverse health effects of heavy metals have long been known, exposure to heavy metals continues and is even increasing in some areas, especially in less developed countries. The main threats to human health from heavy metals are associated with exposure to Pb, Cd, Hg and As. Heavy metal exposure may cause headaches, irritability, abdominal pain, kidney damage, skeletal damage, acute pulmonary effects, cardiovascular disease, chronic renal failure, cancer, lung damage, neurological and psychological symptoms, nervous system damage and much more (Järup 2003). Heavy metals as soil contaminants typically occur in low concentrations (10,000 mg kg^{-1} dry soil). Inorganic material in general does not exhibit characteristic absorption features in the VNIR-SWIR wavelength region. It is therefore considered impossible to directly detect inorganic soil contaminants in general, and heavy metals in particular (Winkelmann 2005). The conventional method of estimating the spatial distribution of heavy metals is by raster sampling and a time-consuming laboratory analysis, followed by geostatistical interpolation (Kemper & Sommer 2002). While pure metals do not absorb in the VNIR-SWIR region, they may be detected indirectly via their complexing with organic matter (OM), association with moieties such as hydroxides, sulfides, carbonates, or oxides which are detectable, or adsorption to clays that absorb light in this wavelength range (Malley & Williams 1997). To explain possible variations in spectral signals due to heavy metals bound to minerals, it is necessary to consider the binding reaction of the metal onto the mineral surface. This approach starts from the premise that the spectrally assigned position of minerals can change with chemical composition and surface activity (Ben-Dor et al. 1999). Despite the occurrence of otherwise similar minerals in different samples, variations in spectral features (e.g., shifts in peak wavelength) may occur according to the nature of highly enriched cations in the mineral. The surface complexation model of the binding reactions of heavy metals describes the binding of metal ions to the mineral surface functional group to form a more stable molecular unit (Christl & Kretzschmar 1999; Zachara & Westall 1999). The main surface functional groups are inorganic hydroxyl groups that bind to surface Al, Fe, Mn, or Si on oxides or Al and Si on the edges of clay minerals (Sparks 1995; Zachara & Westall 1999; Choe et al. 2008).

3.2 Reflectance spectroscopy of heavy metals

The first report of quantitative prediction of heavy metals in sediments by NIRS was published in 1997 by Malley and Williams. They showed the feasibility of predicting six of the seven metals examined (Cd, Cu, Pb, Zn, Ni, Mn, Fe) with NIRS in a highly variable set of sediment samples, collected at Precambrian Shield Lake in northwestern Ontario, Canada. The metals were modeled by both MLR and PLS, and OM was found to be responsible for the prediction of sediment heavy metal concentrations (Malley & Williams 1997). A study published in 2002 by Kemper and Sommer investigated the ability to predict heavy metals following a mining accident that caused an area to be flooded with pyritic sludge contaminated with high concentrations of heavy metals: 214 soil samples were collected, and prediction of heavy metals was achieved by MLR and ANN approaches. It was possible to predict six out of nine elements (As, Fe, Hg, Pb, S, Sb) with high accuracy. Correlation analysis revealed that most of the wavelengths that were important for the prediction could be attributed to absorption features of Fe and Fe oxides (Kemper & Sommer 2002). The study by Kemper and Sommer continued with the collection of an additional 132 soil samples and acquisition of hyperspectral data with the HyMap airborne sensor (Cocks et al. 1998). A spectral mixture modeling approach was applied to the field and airborne hyperspectral data. VMESMA was used to estimate the quantities and distribution of the remaining tailing material. The semi-quantitatively determined abundance of residual pyritic material in the sludge could be transformed into quantitative information for an assessment of acidification risk and distribution of residual heavy metal contamination based on an artificial mixture experiment prepared with three different soils and pure sludge. Unmixing of the HyMap images allowed identification of secondary minerals of pyrite as indicators of pyrite oxidation and associated acidification (Kemper & Sommer 2003). The first study on NIRS of heavy metal contamination in agricultural soils was reported by Wu et al. (2005): 120 soil samples were collected in the Nanjing region, and Hg concentration was modeled by PCR. Correlation analysis revealed that Hg concentration is negatively correlated with soil reflectance, while positively correlated with the absorption depths of goethite at 496 nm and clay minerals at 2,210 nm, suggesting Hg sorption by clay-size mineral assemblages as the mechanism by which to predict spectrally featureless Hg in soils (Wu et al. 2005). In later work, Wu et al. (2007) also explored a physicochemical mechanism that allows estimation of heavy metals with the reflectance spectroscopy method, and concluded that correlation with total Fe (including active and residual Fe) is the major mechanism (Wu et al. 2007). Choe et al. (2008) explored the possibility of extending the use of spectral variations associated with heavy metal parameters to map the distribution of areas affected by heavy metals on HyMAP data in the Rodalquilar gold-mining area in southeast Spain. The chosen spectral parameters showed significant correlations with concentrations of Pb, Zn and As. Later, Choe et al. (2009) examined the potential use of spectral absorption feature parameters to quantify heavy metals using stepwise MLR (SMLR) and enter MLR (EMLR).

Contradictory conclusions on the major absorption feature mechanisms that can enable indirect prediction of heavy metals were presented by the abovementioned studies. However, their results cannot be generalized, as soil properties (e.g., soil C_{org} content) were distinctly different in the different studies (Vohland et al. 2009). Vohland et al. (2009) used soil samples taken from grassland plots that showed a very wide range of both C_{org} (0.37%–9.24%) and clay contents (0%–37.8%), with the aim of investigating whether the heavy metal contents of these samples can actually be assessed by NIRS. In addition, they explored statistically whether a valid prediction mechanism can be identified. According to the correlation spectra and PLS coefficients, it was concluded that the active C_{org} fraction is the key variable for an indirect spectroscopic approach (Vohland et al. 2009). Another study investigating the binding form of heavy metals with agricultural soil constituents was conducted by JiA et al. (2010). The results showed that Cr, Cu, Zn and As have stronger negative correlation coefficients with the absorption features of Fe oxides, clays and OM, However, Cd, Pb and Hg only displayed a significant correlation with the spectral region related to organic carbon (JiA et al. 2010). Pandit et al. (2010) showed very high correlation coefficients between laboratory-determined abundance and the abundance predicted from PLS calibration for several heavy metals, with the correlation coefficient for Pb being the highest of all metals (R=0.992). Soil Pb contamination exhibited a weak correlation (0.473) with percentage of OM, suggesting that while OM is a factor in Pb retention, it cannot be used to determine the quantity of Pb because only a little OM is needed to retain Pb (Pandit et al. 2010).

3.3 Conclusion

Heavy metal elements cannot be detected with reflectance spectroscopy at concentrations under 1000 mg kg^{-1} (Wu et al. 2007). The presence of Fe in ferric or ferrous forms results in absorption features at wavelengths in the 0.4 to 1.3 μm region. A doublet absorption feature near 496 nm is caused by goethite. Two distinct absorption bands at 1,410 and 1,910 nm are attributable to vibrational frequencies of OH groups in the adsorbed water, and the absorption feature at around 2,210 nm is related to OH groups in the crystal lattice water. For pure goethite, two absorption bands, at 670 and 950 nm, are also characteristic and even stronger than the 496 nm band. However, the soil matrix effect prevents these two bands from being displayed in soil spectra (Wu et al. 2005). The intercorrelation between heavy metals and total Fe (including active and residual Fe) is a major predictive mechanism (Wu et al. 2005). Later, Wu et al. (2007) showed that all heavy metals that have a high correlation with Fe also have high cross-validation statistics. The three siderophile elements Ni, Cr, and Co had the highest correlation coefficients with Fe, and prediction accuracy for these elements was also the highest among the eight elements studied. The most poorly predicted element, Cd, showed the lowest correlation with Fe. Another important soil constituent, OM, could absorb electromagnetic waves and adsorb heavy metals; however, the correlation between OM and heavy metals was very poor in the study area (Wu et al. 2007). This is different from results obtained using reflectance spectroscopy in food and forage crop research, where the content of inorganic elements is low relative to the organic matrix. Thus, successful prediction of inorganic components in forage crops can be based on the correlations between inorganic components and organic constituents (Siebielec et al., 2004). Choe et al. (2008) showed that despite the requirement for further corrections, the considered spectral absorption feature parameters had the potential to detect heavy metals. Moreover, the image-derived spectral parameters themselves showed a capacity to screen areas affected by heavy metals as a preliminary observation in determining sampling strategies and precise analyses for the investigation of environmental pollution (Choe et al. 2008). While weak prediction performance might result from the occurrence of many overlapping bands and the limited number of molecules that respond spectrally in the VNIR-SWIR range, a spatial distribution map of heavy metals by EMLR shows a distribution pattern similar to that on a map of measured values (Choe et al. 2009). However, the partial trade-off for time saved is reduced accuracy. Thus, spectral assessment of soil samples cannot replace, but rather complements the classical chemical analysis. The benefits are the practicable processing of a large number of samples and the savings on chemicals, lengthy and tedious processes and manpower. However, a calibration using information obtained from chemical analyses is mandatory (Vohland et al. 2009). In terms of spatial analysis, an airborne or space borne hyperspectral sensor may be useful for the screening of large areas and reproduction of the spatial distribution patterns.

3.4 Summary table

The results of the previously mentioned NIRS prediction models of heavy metals are summarized in Table 1. Different modeling techniques as well as various preprocessing methods were employed in the development of these models, for an in depth description of the models please refers to the proper references.

Authors	Contaminant (R^2)	# of Samples	Modeling Method
Malley & Williams (1997)	Cd(0.63), Cu(0.91), Fe(0.86), Mn(0.93), Ni(0.88), Pb(0.81), Zn(0.93)	169	MLR / PLS
Kemper & Sommer (2002)	As(0.84), Cd(0.51), Cu(0.43), Fe(0.72), Hg(0.96), Pb(0.95), S(0.87), Sb(0.93), Zn(0.24)	214	MLR / ANN
Kemper & Sommer (2003)	As(0.73), Pb(0.73)	346	polynomial fit
Siebielec et al. (2004)	Cd(0.54), Cu(0.61), Fe(0.87), Ni(0.84), Pb(0.45), Zn(0.67)	70	PLS
Wu et al. (2005)	Hg(0.48)	120	PCR
Wu et al. (2007)	As(0.72), Cd(0.20), Co(0.80), Cr(0.85), Cu(0.67), Ni(0.81), Pb(0.55), Zn(0.56)	61	PLS
Choe et al. (2008)	As(0.88), Pb(0.61), Zn(0.60)	49	EMLR
Choe et al. (2009)	As(0.60), Cu(0.81)	22	EMLR
Ren et al. (2009)	As(0.62), Cu(0.41), Fe(0.78)	33	PLS
Vohland et al. (2009)	Cu(0.75), Fe(0.84), Mn(0.71), Pb(0.76), Zn(0.81)	149	PLS
Jia et al. (2010)	As(0.30), Cd(0.10), Cr(0.68), Cu(0.46), Hg(0.15), Pb(0.26), Zn(0.40)	122	Linear Regression
Pandit et al. (2010)	Cd(0.43), Cu(0.81), Mn(0.81), Pb(0.75), Zn(0.76)	8	PLS

Table 1. Heavy metals prediction via NIRS.

4. Petroleum hydrocarbons

4.1 Introduction

Petroleum hydrocarbons (PHCs) are environmental contaminants of great significance. With production rates of 13.42 million cubic meters of crude oil per day (Energy Information Administration 2009), their potential as soil and water contaminants is apparent. Crude oil and refined crude oil products therefore appear in almost every location on earth at the different stages of crude oil production, handling, transport, refining and consumption. The widespread use of PHCs for transportation, heating and industry has led to the release of these fuels into the environment through accidental spills, long-term leakage, and operational failures. Consequently, many soil and water areas are contaminated with PHCs.

PHCs are well known to be neurotoxic to humans and animals. They have been found to affect brain activity and development, and cause many other symptoms including, but not limited to: nausea, disorientation, mental confusion, speech slurring and memory disorders. Exposure to higher levels can cause extreme debilitation, loss of righting, coma, seizures and death. Long-term exposure has been shown to cause changes in neurophysiological or psychological capacity, and to increase the risk of lung, skin and bladder cancers, as well as having other carcinogenic effects (Hutcheson et al. 1996); (Boffetta et al. 1997); (Ritchie et al. 2001). Due to its toxicity, several environmental regulations have been enforced worldwide to protect the environment from PHCs contamination. As a result, attention has also been devoted to rehabilitating PHCs-contaminated soils. For both the diagnosis of suspected areas and the possibility of controlling the rehabilitation process, there is a great need to develop and implement a method that can rapidly detect small amounts of PHCs in soils as well as identify PHCs type, for better soil management and maintenance as a source of life and food.

4.1.1 Total PHCs

Various fuel products are the outcome of crude-oil refinement. These products contain mostly C (83-97%), H (10-14%), N (0.1-2%), O (0.0005-1.5%), S (0.05-6%) and various types of metals such as V, Fe, Cu and so on (<0.1%). These fuel products are classified by two main criteria: structure of the hydrocarbon (aliphatic or aromatic), and number of C in the chemical structure. These two criteria affect several of the products' characteristics, such as boiling point, solubility, absorption and toxicity. There are some overlapping characteristics among the various groups of hydrocarbons but in general, aromatic compounds are more toxic than aliphatic structures and there is a connection between the length of an aliphatic molecule and its toxicity (Tetra Tech EM Inc. for EPA 2000). Total PHCs (TPH) is a commonly used measurement index for quantifying environmental contamination originating from fuel. Choosing an analytical method for assessing TPH in soil samples is a problematic task due to the complex nature of the fuel ingredients and the fact that those ingredients change with time. Today, the most commonly used analytical method for assessing TPH in soil samples is based on the no-longer approved Environmental Protection Agency (EPA) method 418.1. This method was originally intended for assessment of TPH in water (EPA 1978), but was later adjusted to assess TPH in soil samples (EPA 1983). Not only has this previously approved method been withdrawn by the EPA, but it is also problematic for various other reasons.

4.1.2 Analytical methods

4.1.2.1 EPA method 418.1

The most common method in use today, which is based on the EPA's method 418.1 which was adjusted for soils, is costly and time-consuming. Briefly, the sample is brought to the laboratory and a specific extraction technique is applied using Freon 113 (trichlorotrifluoroethane). The TPH level is then determined in the solvent by Fourier transform infrared (FTIR) spectrophotometer. This method necessitates careful shipping of the samples to the laboratory (e.g., cooling conditions and use of special quartz tubes) and several replications to precisely establish the chemical analysis. Measuring one sample can cost about $50 and the results cannot provide information in a near-real-time domain. Consequently, in obtaining a reliable spatial pattern of the contaminated area, many samples are required to generate useful information that will facilitate the rehabilitation or precautionary processes. This method can only be used for quantification of TPH and not for identifying PHCs type.

4.1.2.2 Gravimetric

Hydrocarbons are removed from the sample by an extraction solvent, the solvent is weighed before and after the extraction, and the difference in weight expresses the TPH. This method is analogous to weighing a battleship with its captain, then subtracting the weight of the battleship in order to find out the weight of the captain: while it is relatively inexpensive, it is highly inaccurate.

4.1.2.3 EPA method 8440

Hydrocarbons are extracted from the sample with CO_2 at very high pressure (340 atm), at $80^{o}C$ for 30 min. After extraction, TPH is determined by FTIR spectrometry and compared to a calibration model (similar to method 418.1). This method is not only expensive but it is also dangerous due to the high pressures involved.

4.1.3 TPH field methods

The abovementioned laboratory methods are costly and time-consuming, and usually cannot be implemented in a real-time domain at the contaminated site. Samples need to be transported to the laboratory in a cooled container and there is a waiting period before results are reported. In addition to the laboratory tests, a few field-measurement kits are available. The seven field-measurement devices listed below were tested at the Navy Base Ventura County site in Port Hueneme, California by Tetra Tech EM Inc. (2000):

- CHEMetrics, Inc.: RemediAid™ Total Petroleum Hydrocarbon Starter Kit
- Wilks Enterprise, Inc.: Infracal® TOG/TPH Analyzer, Models CVH and HATR-T
- Horiba Instruments, Inc,: OCMA-350 Oil Content Analyzer
- Dexsil® Corp.: PetroFLAG™ Hydrocarbon Test Kit for Soil
- Environmental Systems Corp.: Synchronous Scanning Luminoscope
- siteLAB® Corp.: Analytical Test Kit UVF-3100A
- Strategic Diagnostics, Inc.: EnSys Petro Test System

These field tests, making use of several technologies, are summarized in Table 2.

Technology	**Measurement Device**
Friedel-Crafts alkylation reaction and colorimetry	RemediAid™ Total Petroleum Hydrocarbon Starter Kit
Infrared analysis	Infracal® TOG/TPH Analyzer, Models CVH and HATR-T; OCMA-350 Oil Content Analyzer
Emulsion turbidimetry	PetroFLAG™ Hydrocarbon Test Kit for Soil
Ultraviolet fluorescence spectroscopy	Synchronous Scanning Luminoscope; siteLAB® Analytical Test Kit UVF-3100A
Immunoassay and colorimetry	EnSys Petro Test System

Table 2. Technology of TPH field-measurement kits

These technologies are thoroughly discussed in the report from the EPA field test conducted in June 2000: while some of these tests produced good results, the kits were found to be expensive and to require a knowledgeable operator (Tetra Tech EM Inc. for EPA 2000).

All analytical methods, whether conducted in the laboratory or *in situ*, that are available for the determination of TPH in soil samples have individual drawbacks, for example: shipping of the sample to a distant laboratory (no *in-situ* availability), long waiting periods for results, high cost per sample, inaccuracy, and the requirement of a skilled staff to perform the measurement. Due to these drawbacks, a new low-cost *in-situ* method for assessing PHC contamination, particularly at a new site, is greatly needed. Emphasized desired properties of this new method are cost-effectiveness and its ability to be implemented by not only highly trained personnel.

4.2 TPH and reflectance spectroscopy

4.2.1 Absorption features of organic compounds

Carbon-hydrogen bonds (C-H, $C\text{-}H_2$, $C\text{-}H_3$), hydroxy groups (O-H), double and triple bonds of aliphatics and aromatics, carboxyl groups (C=O), ester groups (C-O-C), amino groups (N-H) and other structural groups of organic chemicals exhibit characteristic fundamental vibrations that are evident in the 2,500 to 6,670 nm wavelength region of the IR spectrum. Furthermore, these compounds exhibit overtone and combination bands in the IR and VNIR-SWIR spectrum between 500 and 6,670 nm. These fundamental vibrations can be determined by IR spectroscopy and some of the overtone and combination bands can be determined by VNIR-SWIR spectroscopy (Winkelmann 2005).

4.2.2 VNIR-SWIR spectroscopy of organic chemicals

As IR spectroscopy is the better choice for qualitative analysis, VNIR-SWIR spectroscopy is primarily applied for quantitative analysis in many disciplines (termed “chemometrics”). However, despite the fact that VNIR-SWIR spectra of organic chemicals are often highly complex due to many overlapping overtone and combination absorption features, they still contain information on the functional and structural groups of the chemicals under investigation. During the past 10 years, a new quantitative methodology has been widely developed that utilizes the reflected radiation in solids. Basically, this technology was adopted 40 years ago from a strategy developed in the food science discipline (Ben-Gera & Norris 1968a; Ben-Gera & Norris 1968b), whereas today it is widely utilized in many industrial and scientific applications. In this approach, the radiation reflected across the

VNIR-SWIR region is modeled against constituents determined by traditional chemical analysis and then used to predict unknown samples. This technology permits rapid and cost-effective measurements on many samples at a given location and therefore significantly reduces chemical analysis costs. Despite the heterogeneity of the methodology, it was demonstrated by many (e.g: Nanni & Demattê 2006; Ben-Dor, et al. 2008; Rossel & Behrens 2010) that soil constituents can be extracted from a simple reflectance measurement (laboratory and field) if an appropriate calibration model has been previously developed. Since Ben-Dor et al. (1995)'s pioneering study, many other workers have explored this promising technology for soils and a significant breakthrough in this area was its replacement of wet chemistry in many scenarios. The adoption of this approach to evaluate hydrocarbon contamination in soil is obvious and a few workers have partially demonstrated this application, as described in the next section. As shown above, VNIR-SWIR spectroscopy holds great potential for quantitative chemical analysis. If calibration standards are used, the identification of known substances in a mixture and the determination of their concentrations are possible. Furthermore, it seems likely that the identification of substances based on their VNIR-SWIR spectra will become possible for classification purposes.

4.3 Reflectance spectroscopy of PHCs

The spectral properties of hydrocarbons were identified in the late 1980s, although it has been argued that these properties were only visible at concentrations of 4% wt (by weight) and above (Cloutis 1989). In the mid 1990s, a VNIR-SWIR reflectance sensor was developed as a proof of concept for the detection of OM in soil based on the same spectral properties identified by Cloutis. The sensor was designed for the detection of benzene in soil at a minimal concentration of 4.4% wt: several configurations were tested and minimal information was provided (Schneider et al. 1995). Soon after, the U.S. Department of Energy contracted a private company to investigate the application of reflectance spectroscopy to determine motor oil contamination in sandy loam. A schematic design for a field instrument was suggested, although only one contaminant and one type of soil were tested, using very few samples with a very limited range of contamination (Stallard et al. 1996). A more inclusive study was conducted shortly thereafter, using three types of soil contaminated in the laboratory with diesel and gasoline. A 0.1% wt and 0.5% wt minimum detection limit was achieved, respectively (Zwanziger & Heidrun 1998). The first study utilizing field-collected samples was not able to produce robust models, resulting in a very low correlation coefficient ($R^2 = 0.46$) and large errors, probably due to very low sample count and problems with the chemical measurements performed in the laboratory which produced inconsistent results (Malley et al. 1999). Attempts at mapping hydrocarbons using Landsat and Daedalus in 1994 and 1995 failed, probably due to the limited spectral resolution of the sensors (multispectral sensors); on the other hand, the higher spatial and spectral resolution, as well as the very high signal-to-noise ratio of the airborne hyperspectral sensor used (HyMap) (Cocks et al. 1998) yielded successful identification of hydrocarbon- and oil-contaminated soils, but only for high contaminant concentrations (2.5% wt) (Hörig et al. 2001). Based on the HyMap mission, a Hydrocarbon Index was developed for the mapping of hydrocarbon-bearing materials. This index is limited to very high signal-to-noise ratio sensors, as well as by other issues such as problems with land cover, vegetation and high concentration detection levels (Kühn et al. 2004). The most comprehensive work on reflectance properties of hydrocarbons was conducted by Winkelmann (2005): several types of hydrocarbons were mixed with several types of soil under laboratory conditions. They were measured spectrally and an attempt was made to separate them into hydrocarbon groups using the reflectance spectra; hyperspectral airborne remote sensing was also applied to identifying hydrocarbon contamination. No quantitative models were tested, although this was mentioned as an avenue of further study (Winkelmann 2005). A recent study by Chakraborty et al. (2010) on the prediction accuracy of VNIR-SWIR reflectance spectroscopy of petroleum contaminated soil, showed fair validation results (R^2=0.64). The study included 46 field collected samples that were preprocessed and modeled by several techniques.

While the abovementioned studies addressed only concentration levels >0.1%, today's environmental regulations require identification levels that are an order of magnitude lower. Moreover, none of the above studies dealt with identifying PHCs type and mixtures in soil using reflectance spectroscopy. A comprehensive study including several types of soil with several types of PHCs at a large variety of contamination levels has not yet been published. Most of the studies were performed with lab-prepared samples and almost none collected in the field. Furthermore, no generic models were developed, especially combining laboratory and field samples, and no real quantitative operation model was presented for real-life applications.

A long-term study conducted by Schwartz (2012) over the past 3 years included the development of NIRS models for the prediction of TPH in soil samples at high precision for low contamination levels, as well as PHCs classification. Five types of soil were mixed with three types of PHCs at 50 concentration levels, giving 750 laboratory samples, along with more than 200 other laboratory-prepared samples with various mixtures to study the soil properties' effects on spectral measurements and chemical analysis. The samples were evaluated for TPH level with EPA method 418.1, as well as measured spectrally by an ASD Fieldspec Pro. An "all possibilities" approach was taken for the creation of robust NIRS models, including the evaluation of many preprocessing techniques (smoothing, absorbance, first and second derivatives, and continuum removal), as well as PLS and ANN modeling methods. Forty-one soil samples were then collected in a field campaign and TPH level was predicted by both the PLS and ANN models, as well as being determined by the traditional EPA 418.1 method. A detection limit of 1,000 ppm was set, and samples with concentrations of 1,000 ppm or lower (27 samples) were successfully predicted as non-contaminated or with very low contamination. The rest of the predicted samples gave a correlation between the predicted and measured values of $R^2 > 0.9$ (this study will be published soon as a Ph.D. thesis, and as several papers in peer-reviewed journals).

5. Acid mine drainage

Acid mine drainage (AMD) from mine wastes is considered a major problem in mining areas, because of the risk of heavy metal contamination of soils and water, Reflectance spectroscopy and NIRS is also used in the field of AMD. AMD is a byproduct of the oxidation of iron disulfide mineral (pyrite). While AMD reacts with the surrounding rocks and soil, heavy metals become more soluble and they can leach into the environment. This process is also substantially enhanced by bacterial action. In mining areas, the release of heavy metals is accelerated due to the increased oxidation rates, which are caused by the greater accessibility to air through mine workings, waste rock, and tailings by mineral processing. Furthermore, there is the added risk of mining accidents such as in Aznalcóllar

(Spain 1998) or Baia Mare/Borsa (Romania 2000). During the course of these accidents, huge amounts of mine waste and toxic substances (heavy metals, cyanide) were set free in one go, contaminating rivers and alluvial soils (Kemper & Sommer 2002). Pyrite oxidation is one of the most acid-producing natural weathering processes, in which trace metals are mobilized and released into the river system and groundwater. It is a complex process that proceeds rapidly when pyrite is exposed to air. In a first step, pyrite produces a solution of ferrous sulfate and sulfuric acid. The dissolved ferrous iron continues to oxidize and hydrolyze, producing additional acidity. During the oxidation process, the pyrite transforms first to copiapite, then to jarosite, schwertmannite, ferryhydrite and eventually to hematite or goethite (Kemper & Sommer 2003).

Based on the sulfur content, other information can also be derived. Sulfur plays a key role in the assessment, because it is one of the two elements forming pyrite (FeS_2), which is the main component of the sludge (75-80% wt), and it is the main source of acidity when pyrite oxidizes. The sulfur content is also used in conventional mining waste analysis for the prediction of its geochemical behavior and to identify wastes that are likely to be acid-generating or susceptible to heavy metal leaching (Kemper & Sommer 2003).

6. Pesticides

Soil contamination by agricultural pesticides is a matter of increasing concern. Several authors have reported accurate predictions of pesticide sorption to soil using NIRS. Bengtsson et al. (2007) used NIRS to predict lindane and linuron sorption to soil samples. The pesticide-content predictions were established by PLS of the VNIR-SWIR spectra and sorption data obtained using the batch equilibrium technique. PLS could predict 85% of the soil sorption (Kd) for lindane and 84% for linuron, respectively. Later, Brunet et al. (2009) tested the feasibility of determining chlordecone contamination in soils. Chlordecone, also known as kepone, is a chlorinated polycyclic ketone pesticide. It is toxic, persistent, and induces cumulative and delayed toxicity. The regular method to measure chlordecone involves extraction with two solvents and quantification by gas chromatography–mass spectroscopy. A total of 236 samples were collected at 0–30 and 30–60 cm depths in Andosols, Nitisols and Ferralsols from all over the eastern part of Martinique (French West Indies). Chlordecone content was determined by wet chemistry and the reflectance spectra were recorded. Modified PLS (mPLS) regression was used to correlate spectral data to conventional values. While conventional measures and NIRS predictions correlated poorly at high chlordecone contents (>12 mg kg^{-1}), when the validation set was divided into four classes based on chlordecone content, NIRS prediction properly classified 80% of the samples (Brunet et al. 2009).

7. Vegetation stress as an indicator for soil contamination

The concept of using plants as indicators for soil contamination has been tested for many years, for many good reasons: 1) inorganics in general do not exhibit characteristic absorption features in the VNIR-SWIR wavelength region; 2) the plant's root system is susceptible to contaminated material, and extracts and transports it to the aboveground plant parts, and 3) soil is far from always being bare; it is often covered with plants. Free metal ions in the soil solution and exchangeable ions of clays and OM are mostly available for plant uptake (Lasat 2000). For their development, plants require not only macronutrients

(N, P, K, S, Ca, and Mg), but also essential micronutrients such as Fe, Zn, Mn, Ni, Cu, and Mo. Most of these micronutrients accumulate in the plant tissues for their metabolic needs, but they never exceed 10 ppm. Higher levels of these metals (and other heavy metals) in plant tissue might have phytotoxic effects, sometimes resulting in death (Winkelmann 2005). Vegetation stress caused by heavy metal toxicity can show different symptoms, depending on the heavy metal's type and concentration, and on the plant species' sensitivity. The phototoxic effects typically cause physiological, morphological and ecological changes, expressed in many different ways, from chlorosis (reduced production of chlorophyll and hence reduced photosynthetic activity), leaf wilting, needle retention, branch density reduction, defoliation, growth inhibition, flowering and fruiting changes, dwarf growth and gigantism, to changes in plant species distribution, the establishment of adapted species, and the dying-off of whole plants or communities (Winkelmann 2005). However, stress can be induced in vegetation by a large number of other factors, including water deficiency, poor soil drainage, poor soil aeration, soil salinity, weed competition, pest infestation, nutrient deficiency, or nutrient poisoning (Levitt 1980; Lichtenthaler 1996).

A large number of studies have reported on spectra of metal-stressed vegetation being clearly different from those of unstressed vegetation. These differences usually include decreases in both NDVI and RVI with increasing plant stress (Davids & Tyler 2003; Dunagan et al. 2007), signs of reduced biomass and a shift of the red edge position (REP) (Kooistra et al. 2004; Dunagan et al. 2007) or red edge slope (Zhou et al. 2010), signs of reduced photosynthetic activity due to chlorosis and decreased reflectance in the 700 nm to 2,500 nm wavelength region (Kooistra et al. 2003). Some heavy metals which are considered contaminants are also essential micronutrients for all vegetation species, in particular Cu and Zn. Therefore, in some cases, even positive effects in reflectance spectra can be observed when one of the elements under investigation is clearly a micronutrient with limited availability. Such effects include a red-shift of the red edge and decreased reflection in the VIS wavelength region indicating increased photosynthetic activity (Horler et al. 1980). As no vegetation stress symptoms and corresponding spectral characteristic could be related specifically to heavy metal stress or any other contaminant stress, the sources causing the observed stress need to be carefully identified and separated.

8. Limitations, obstacles and problems

8.1 Laboratory

A wide range of factors can affect soil reflectance spectra in both laboratory and field domains. In the lab, different spectrometers, or even repeating a specific sample's measurements in the same spectrometer may result in variations. Such variations might include subtle or strong alterations in wavelength location, peak absorption shape or radiometric intensity. In addition to the instrumentation itself, internal electronic noise can affect the measurements and mechanical noise factors (e.g. homogeneity and purity of the white reference panel, or subtle movement when holding the fiber optic) can strongly affect their consistency. In soil samples, where very weak spectral features are monitored for chemometric purposes, these noise factors can alter the robust use of a selected spectral model for a wide range of spectrometers and users. Recently, Pimstein et al. (2011) examined the variation stemming from the above sources using three ASD Fieldspec Pro spectrometers and developed a standard protocol for laboratory spectral operation. They also suggested using the same internal standard worldwide in order to correct the spectra of any spectrometer to a selected master spectrometer. They demonstrated that chemometric analytical results are more coherent and precise. A global soil spectral library, not just as with minerals, has to be followed by chemical information. Recently, Viscarra Rossel (Rossel, Soil Spectroscopy Group 2009) initiated a spectral archive containing more than 10,000 spectra taken worldwide and is in the process of integrating them into this library with some major soil attributes measured in the alternative "wet chemistry" way.

8.2 Field

In the field, there are more uncertainties than in the laboratory, such as atmosphere attenuation, sun angle, aspect and slope of the sample area, large pixel sample, BRDF effects and most of all, soil crusting and sealing which can emerge on any soil surface. The latter prevent representation of the real soil body in the measurement. In the laboratory, the soil is crushed to pass a 2 mm sieves, and all stones and litter debris are consequently removed before the measurement. In the field, those materials are present, as are physical and biogenic crusts and possible dust contamination. Another problem that might emerge in field soil measurement is the adjacency effect and the mixed pixel. The first can occur when the pixel in question is surrounded by bright reflective targets. The mixed pixel problem occurs when a pixel is composed of several chromophores. It is simple when the mixture is binary and complex when it is not. The sun's angle, slope and aspect might not be factors in the field if artificial illumination is used in a standard procedure. A contact probe equipped with tungsten-halogen illumination can be used, with caution. This is because the soil surface measured by such an instrument may consist of a very narrow field of view which might not represent the soil in question, but rather debris, stones or even soil aggregation.

8.3 Airborne and spaceborne

Soil reflectance can also be measured from aircrafts and satellites, using either a point spectroscopy (Karnieli et al. 2001) or IS (Ben-Dor et al. 2009) sensor. However, more difficulties arise during such measurements to extract the correct reflectance, such as: small integration time (less photons), strong atmosphere effects, large pixel size and varying quality of the sensor's stability and sensitivity. Brook & Ben-Dor (2011) have recently developed a more moderate method (SVC—Supervised Vicarious Calibration) to standardize all sensors' radiometric readings, with the aim of deriving the optimal soil reflectance from the airborne IS sensor. This method uses artificial net targets with varying densities placed on a bright background area. It is easy to use and performs well. The method has been recently validated in a European campaign over southern France, using three different sensors simultaneously onboard two different airplanes. The preliminary results were relatively good as compared to the alternative ways of deriving the reflectance from the airborne sensor (unpublished data). The artificial target and the suggested method help assess the atmospheric attenuation, and minimize sensor instability while correcting for the systematic noise. Another limitation using airborne IS for soil is its high cost, low availability, and the complexity involved in processing the raw data into a final reflectance product (Ben-Dor et al. 2009). These factors actually prevent ordinary users from using this technology, classifying it as an exclusive method. Vegetation coverage (partial or total) is also a problem for deriving correct soil reflectance from afar when it is mixed in the sensor's field of view.

As a further step, the retrieval of quantitative mapping using spectral parameters derived from hyperspectral images should take into account differences in the spectral and spatial resolution between ground spectrometer and image data (Choe et al. 2008).

8.4 General issues

In all of the above domains, it must be remembered that reflectance spectroscopy is strongly affected by water content, particle size distribution and the measurement protocol. In addition, ways of calculating the reflectance from radiometric readings, i.e. against a white standard panel or using the radiance-to-irradiance ratio, may change the final product. Furthermore, the reflectance represents only the surface and cannot provide information on the soil profile (unless a penetrating fiber optic is used such as that described by (Ben-Dor et al. 2008). When obtaining a soil spectrum from a user, a meta-data set which characterizes all of these factors (moisture content, grain size and method of reflectance calculation) is strongly needed, because they have a significant impact on the final spectra's behavior.

8.5 Summary

Soil reflectance is an inherent property of the soil but many factors can affect its performance. Internal standards, a standard protocol and controlled conditions are a few of the things that can assist in sharing and comparing soil spectra (and chemometric models) worldwide (e.g. the Global Soil Library by Rossel and Soil Spectroscopy Group (2009)). It is obvious that uncertainties in the laboratory are smaller than those in the field, and the latter are smaller than those obtained from air or space. Use of radiometric data acquired from remote sensing domains to measure soil reflectance information should therefore be undertaken with caution.

9. Future potential of remote sensing technology for assessing soil contamination

Soil reflectance has become a very useful tool over the past 20 years in the laboratory, in the field and from air and space. As the sensitivity of portable field spectrometers increases, field soil spectroscopy is expected to become a basic tool for rapid point-by-point monitoring of the soil environment. The commercial development, operation and use of air- and spaceborne image spectrometers can provide near-laboratory-quality spectra of every pixel in an image and very soon, will permit remote sensing of soils with high standards. Information about soils from reflectance spectra in the VNIR and SWIR spectral regions represents almost all of the data passive solar sensors can provide. It is anticipated that the thermal IR regions will also become part of the soil spectral arena as they contain diagnostic information on some soil attributes that the reflective spectral region does not. The development of a thermal spectrometer (either point or imaging) will enable obtaining more spectral information with better accuracy. Another future insight is the capability to measure the soil profile's spectral distribution using a fiber optic assembly and small boreholes. Today, the major limitation of this technique is the fiber optics' length (as the fiber length increases, the signal across the SWIR region decreases—today, the length is limited to 1.5 m). Miniaturizing the spectrometer will enable placing it on the penetrating optical head, thus bypassing the limitation imposed by fiber length. This, in turn, will enable spectral measurement of any profile depth which, when combined with the NIRS model, might be able to provide *in-situ* soil contamination attributes of the soil profile. Combining the penetrating optical device information with the hyperspectral remote sensing data will also enable extracting the spatial distribution of the contaminant in question in 3D view. Data merging of soil reflectance information with GIS layers and other potential sensors that can be used simultaneously is also a key vision for the future. Time-series spectral measurements of large areas are also very important. Future IS from orbit will enable global coverage of every point on the globe with very good signal-to-noise ratio, such as the PRISMA (Labate et al. 2009) ,HyspIRI (Knox et al. 2010) and EnMAP (Stuffler et al. 2007) programs. This will enable monitoring soil surface changes in short- and long-term scenarios. Another future activity related to soil spectroscopy and soil contamination assessment is the development of better computing systems in which better models to derive highly accurate soil attributes will be achieved. To that end, the "all possibilities" approach in which all statistical and data-manipulation techniques can be applied automatically to a set of data is strongly required. Computing power and simple operation algorithms are key factors to that end.

10. General conclusions and summary

Soil reflectance spectroscopy can assess soil properties rapidly and quantitatively in both point and spatial domains. Direct and indirect soil properties, as well as soil contamination characteristics, can be extracted efficiently at low cost *in situ*. To that end, the VNIR-SWIR spectra must be preprocessed and modeled against reference data obtained by traditional methods. While some soil contaminants are featureless in the VNIR-SWIR region, their detection and quantification is possible, as they may be detected indirectly based on their association with other detectable materials. Although a wide range of factors can affect soil reflectance spectra in both laboratory and field domains thus strongly affecting the consistency of the resulting measurements, recent developments and proper protocols are allowing for more consistent and accurate results. While using NIRS to predict soil contaminants can save time, some cases involve the trade-off of reduced accuracy. Thus, the spectral assessment of soil samples cannot completely replace, but rather complements the classical chemical analysis in these and other cases. The benefits of using NIRS can result in the practicable processing of a large number of samples and savings in chemicals, lengthy tedious processes and manpower. In terms of spatial analysis, an airborne or spaceborne hyperspectral sensor can be useful for the screening of large areas and the reproduction of the spatial distribution patterns of contaminated soil areas. Nevertheless, the field of reflectance spectroscopy as a tool for monitoring contaminated soils still requires further study toward increased accuracy and the development of practical real-life applications.

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5

Multi-Technique Application for Waste Material Detection and Soil Remediation Strategies: The Red Mud Dust and Fly Ash Case Studies

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1. Introduction

Increasing amounts of residues and waste materials coming from different industrial activities have become a serious problem for the future. However, over the last few years there has been a growing emphasis on the utilization of these materials in several remediation technologies in order to clean up contaminated soil.

Among them, two examples of industrial residues are fly ash and red mud.

Fly ash is a by-product of thermal power plants partly used in concrete and cement manufacturing. More than half of it is disposed of in landfills because it finds no other application. It is composed of minerals such as quartz, mullite, subordinately hematite and magnetite, carbon, and a prevalent phase of amorphous aluminosilicate.

Red mud is a waste material formed during the production of alumina when the bauxite ore is subject to caustic leaching. It is mainly characterized by the presence of hematite, goethite, gibbsite, rutile and sodium as sodium aluminum silicates or hydro-silicates. A wide variety of organic compounds could also be found (e.g. polybasic and polyhydroxy acids, humic and fulvic acids, carbohydrates, acetic and oxalic acids, furans).

The mineralogical and chemical characterization of these two waste materials is generally carried out by X-ray powder diffraction, thermal analysis, infrared spectroscopy, scanning electron microscopy and chemical methods. Imaging spectroscopy under controlled conditions in laboratory is also applied.

Many research activities on the neutralization of fly ash and red mud materials as well as to solve the problems connected to their disposal are developed in the last few years. Some of these focus on their utilization in different remediation technologies to immobilize toxic elements. They are in fact used in solidification/stabilization technologies for soil remediation treatment and some studies are based on the immobilization of toxic elements in synthetic zeolites crystallized by treated fly ash.

The chapter investigates these two industrial residues focusing both on their chemical-mineralogical properties and their characterization as toxic materials. Studies of remediation methods to reduce the environmental risks due to polluting metals by using red mud and fly ash are presented as well as examples of landfill monitoring and airborne hyperspectral remote sensing application to analyze red mud soil contamination near urban areas. Significant research activities are being carried out and the aim of this chapter is to show the latest studies underlining the importance of multi-technique application in laboratory and plant scale studies.

2. Fly ash and red mud characterization

2.1 Fly ash

Fly ash is the main combustion by-product from coal-fired power plants and it is partly used in cement manufacturing due to its well-known pozzalonic reactivity (Larosa, 1992). Unfortunately, more than half of fly ash is disposed of in landfills because it finds no other application. The huge production of fly ash is extremely worrying because of this kind of disposal and several investigations have been carried out in order to try to exploit this waste material.

Over the last few years fly ash has been gaining ground in finding solutions to environmental problems and in particular it has being used to the synthesis of zeolites, hydrated aluminosilicate minerals with a three-dimensional open structure making them very useful for solving the mobility of toxic elements in a number of environmental applications. This is due to the mineralogical composition of this waste material.

Fly ash is characterized by quartz, mullite, subordinately hematite and magnetite, carbon, and a prevalent phase of amorphous aluminosilicate (Bayat, 1998; Hall &Livingston, 2002; Hower et al., 1999; Koukouzas et al., 2006; Kukier et al., 2003; Mishra et al., 2003; Sokol et al., 2000). The abundance of amorphous aluminosilicate glass, which is the prevalent reactive phase, is what makes fly ash an important source material in zeolite synthesis.

Fly ash cannot be properly used, both in cement manufacturing and in environmental application, without an in-depth knowledge of its mineralogical and chemical characteristics. So far there have been lots of publications dealing with the morphological characterization of this material using scanning electron microscopy technique equipped with backscattered and secondary electron detectors and coupled with energy dispersive X-ray spectrometer (SEM-EDS) (Katrinak & Zygarlicke 1995; Kutchko & Kim, 2006; Sokol et al., 2000; Vassilev et al., 2004). Many studies have been carried out by using the thermal analysis (TG/DTA) (Hill et al., 1998; Li et al., 1997; Majchrzak-Kuceba & Nowak, 2004; Paya et al. 1998; Sarbak & Kramer-Wachowiak, 2001; Szécsényi et al. 1995; Vempati et al. 1994) and the X-ray powder diffraction (XRD) (McCarthy & Solem,1991; van Roode et al., 1987; Ward & French, 2006) in order to gather compositional information, too. Many works report the use of XRD and Fast Fourier spectroscopy (FTIR) (Vempati et al.1994) in order to identify and quantify glassy materials contained in fly ashes.

Fly ash application is also closely related to its chemical composition. In fact, a large amount of potentially hazardous leachable elements (Brindle & McCarthy; 2006; Jegadeesan et al., 2008; Nakurawa et al., 2007) restricts the application of this material.

2.1.1 Characterization of italian fly ash samples: a case study

The authors characterized four Italian fly ash samples through a multi-method approach. In order to determine the possible utilization of these materials for concrete and cement manufacturing or for environmental application, also synthesizing zeolite and several morphological, chemical and compositional parameters were thoroughly investigated and compared.

Four coal fly ashes resulting from the combustion of four different coal materials were supplied by ENEL thermoelectric powder plants in Brindisi and Venice – Italy.

The particle size distribution was studied by laser granulometry using the principle of laser diffraction. The fly ash samples were also analysed by SEM-EDS. This analysis provided detailed imaging information about the morphology and surface texture of each single particle, as well as the elemental composition of samples.

The chemical abundance of major elements was determined by X-ray fluorescence (XRF) (Franzini et al., 1075; Leoni & Saitta, 1976) and the concentrations of potentially harmful trace were measured by inductively couple plasma spectrometry (ICP-MS) after total acid dissolution treatment of the samples.

The mineral composition of fly ashes was determined by XRD and the quantitative XRD analysis of crystalline phases was carried out by using the reference intensity ratio (RIR) method (Chung, 1974a; 1974b; 1975) combined with the "method of known additions" (Snyder & Bish; 1989). The amount of amorphous materials was calculated through the subtraction of crystalline components. Finally, thermogravimetric analyses were carried out in order to find out the concentration of unburned carbon.

The complete resulting distribution of the particles of fly ash samples are shown in Figure 1. Two samples are mainly made of particles whose diameters range from 5 to 50 μm, the other fly ashes show two main set ranges from 5 to 30 μm and from 70 to 90 μm. All the samples analyzed show an ultra fine fraction ranging from 0.5 to 2.0 μm. This slight variation in size distribution could be due to similar methods for collecting fly ash used in the different power stations.

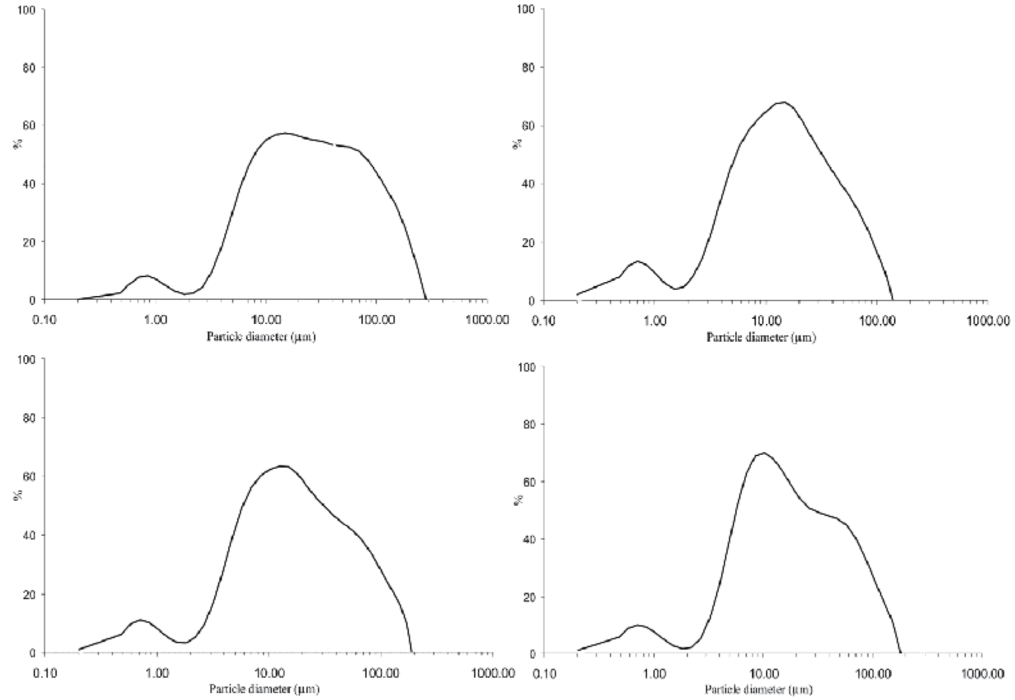

Fig. 1. Particle size distribution of the fly ashes samples.

The morphological study confirms the results on particle distribution and shows that the typical aspect of globules is close to an ideal sphere in shape (Fig. 2).

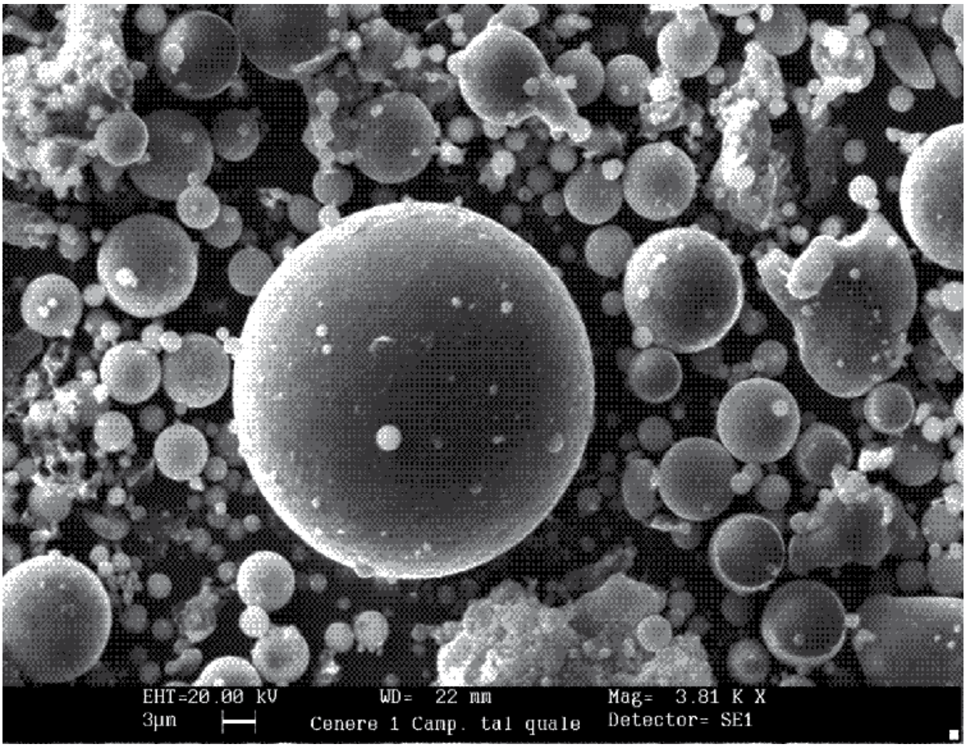

Fig. 2. SEM images of typical fly ash shape.

The amount of unburned carbon was estimated to be about 6% derived by comparing the results obtained through the thermogravimetric analysis carried out in different environmental atmospheres (Fig. 3). LOI value was calculated by using both flow of inert and oxidising gas (the latter is composed of air and CO2, 1:1). When fly ashes were heated in inert gas, the loss of weight due to the water released from hydrated lime can be estimated because, obviously, in an inert environment, carbon oxidation does not take place. The utilization of an oxidising atmosphere permits the determination of the loss of ignition due to carbon oxidation, plus a water loss from hydrated lime. The first reaction takes place within the range 500-800°C, as showed by a large peak in that range. A comparison between the two values permits the calculation of the amount of unburned carbon more precisely.

The XRD patterns in Figure 4 show that the main crystalline phases are mullite and quartz. The broad hump in the region between 10 and 25 °2θ indicates the abundant presence of glassy phase due to the rapid cooling of fly ash at high temperatures. The weight fractions of mineral and amorphous phases are shown in a diagram (Fig. 5).

The high percentage of amorphous material and the presence of hematite/goethite on trace give evidence of the application of these fly ashes in cement products and zeolite synthesis.

The chemical composition for major elements, the SiO_2/Al_2O_3 ratio ranging from 1.7 to 2.0, and the low concentration of toxic elements represent important factors for the application of these materials as well.

Basing on their physical, chemical and mineralogical composition, all the fly ash samples analyzed could be used in cement manufacturing and environmental application.

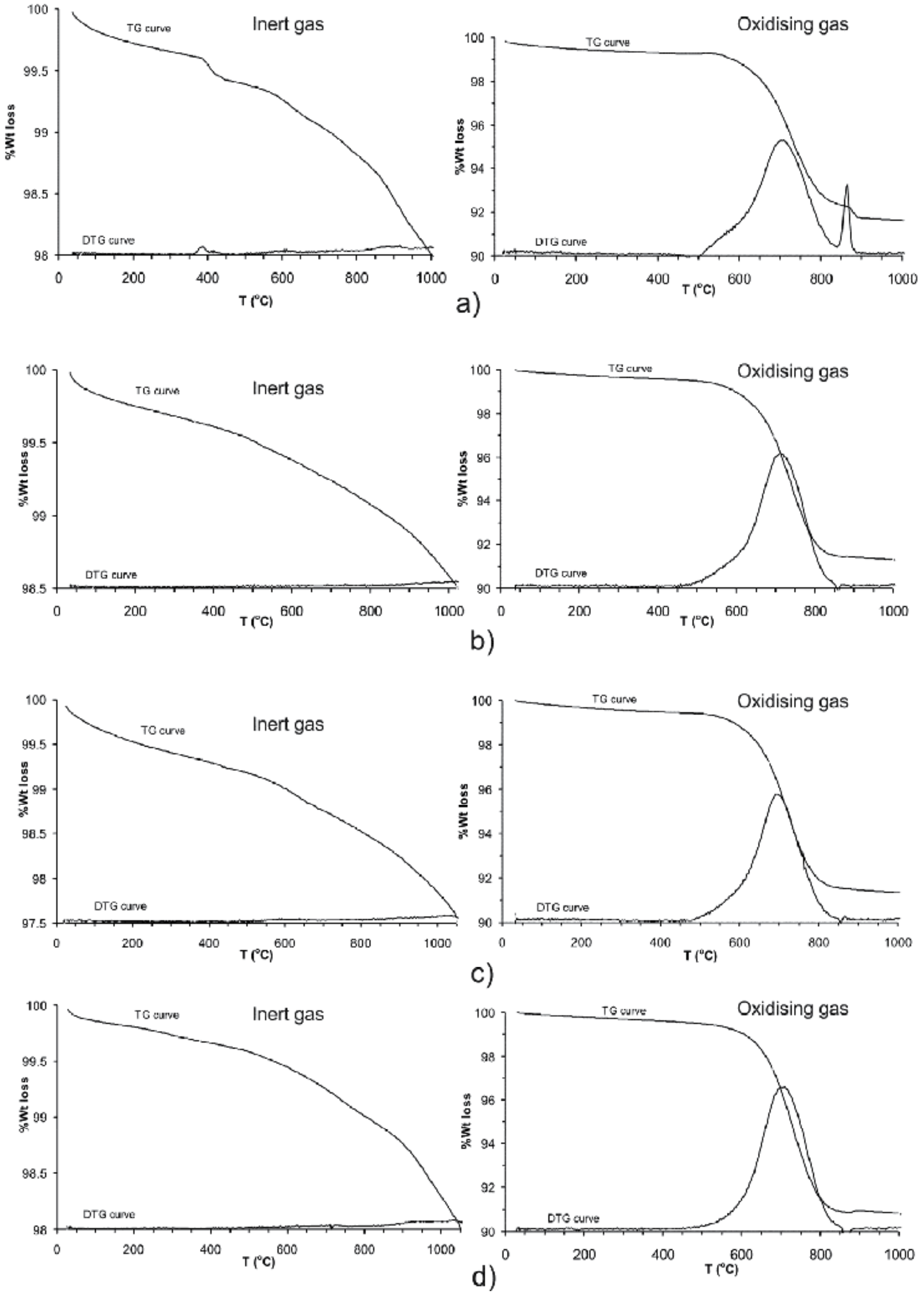

Fig. 3. Thermogravimetric analysis of fly ashes. TG and DTG curves using inert or oxidising gas.

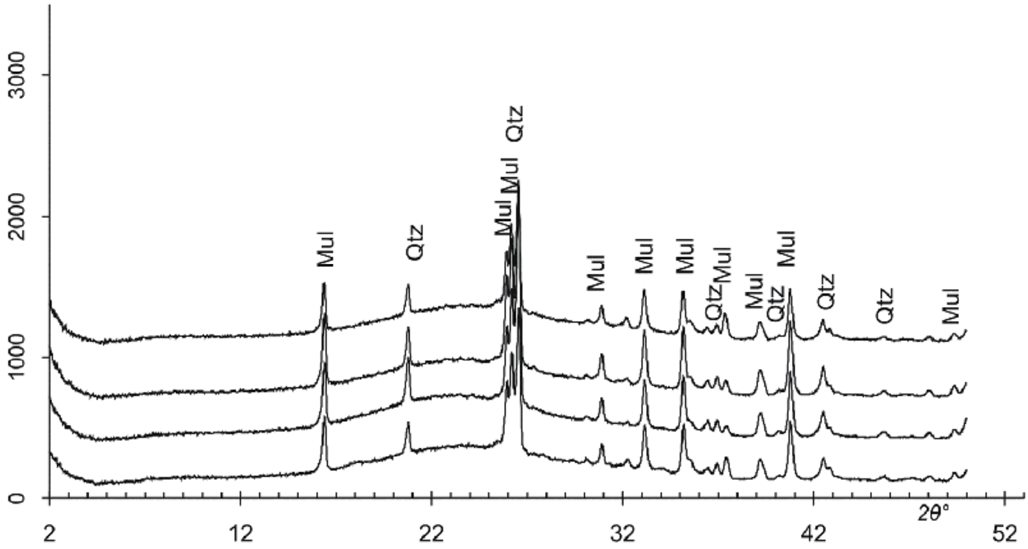

Fig. 4. X-ray diffraction patterns of each samples of fly ash and relative weight fractions of minerals and amorphous phases. Mul = mullite; Qtz = quartz.

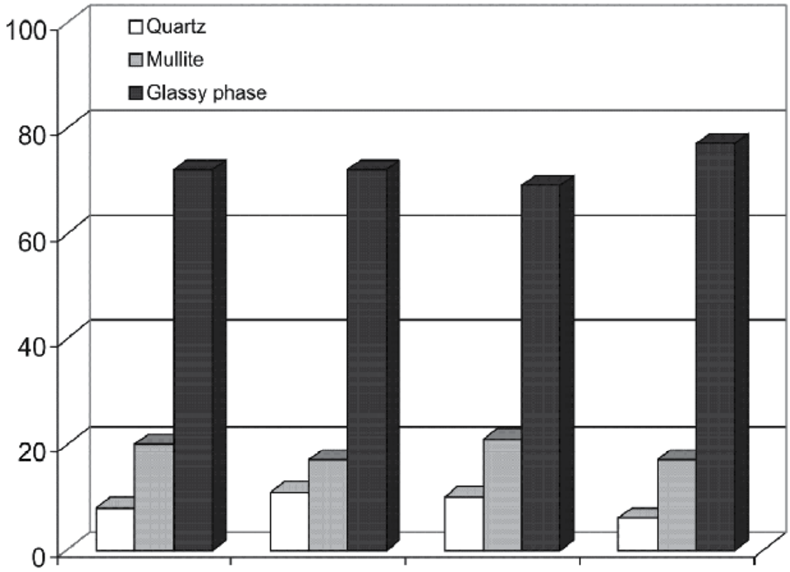

Fig. 5. Weight fractions of minerals and amorphous for each sample of fly ash analyzed.

2.2 Red muds

Red muds are residue alumina products deriving from the Bayer process by the digestion of crushed bauxite in concentrated caustic (NaOH) at elevated temperature. They consist mostly of hematite and goethite together with boehmite, calcium oxides, titanium oxides and alluminosilicate minerals (e.g. Hanahan et al., 2004; Santona et al., 2006). The chemical analysis generally reveals the presence of Si, Al, Fe, Ca, Ti as well as an array of minor constituents such as Na, K, Cr, Ni, Mn, Cu, Zn and Pb (e.g. Chvedov . et al., 2001; Hanahan et al., 2004; Palmert et al., 2007).

Red mud varies in physical, chemical and mineralogical properties due to differing ore sources and refining processes employed and for this reason also this waste material must be deeply characterized before its use for environmental application.

The red mud waste risk is mainly due to the accumulative contamination of land and the surrounding dwellings with fine particulate that is highly alkaline and hence needs special precaution to prevent contamination of surrounding natural or urban environments and to avoid consequential exposure and health risk to inhabitants (Mymrin &Vazquez-Voamonde, 2001).

For this kind of studies, the total element composition is usually analyzed by X-ray fluorescence spectroscopy (XRF), whereas the mineral composition is determined by X-ray diffraction (XRD). The samples are also used for examination of micromorphological characteristics by SEM and for thermogravimetric analysis. Few spectroscopic studies are available (Palmer et al, 2007, 2009) including mid-infrared (IR), Raman, near-infrared (NIR), while there is limited report on the red mud optical characterization.

Recent literature data also show the utilization of imaging spectroscopy and airborne hyperspectral remote sensing to characterize red mud and mapping the red dust distribution on soils (Pascucci et al., 2009). Furthermore, different studies have highlighted the application of field and imaging spectroscopy for identifying minerals and soils containing pollutants (e.g., heavy metals) as an indicator of contamination in mining areas (Choe et al., 2008; Mars & Crowley, 2003). Kemper and Sommer (2002) in their study have been assessed heavy metal concentrations using reflectance spectroscopy and statistical prediction models recommending the opportunity of applying their technique to remote sensing. In Swayze et al. (2000) the authors describe a procedure and their results attained using imaging spectroscopy to map acidic mine waste. Cécillon et al. (2009) in their work examine critically the suitability of NIR reflectance spectroscopy as a tool for soil quality assessment concluding that (a) imaging NIR enables the direct mapping of some soil properties and soil threats, but that further developments to solve several technological limitations identified are needed before it can be used for soil quality assessment and (b) the robustness of laboratory NIR spectroscopy for soil quality assessment allows its implementation in soil monitoring networks, however, its regular employ requires the development of international soil spectral libraries that should become a priority for soil quality research.

2.2.1 Hyperspectral remote sensing data for mapping red dust: a case study

Techniques for direct identification of materials through the exploitation of spectral features from field and laboratory reflectance spectra have been in use for many years being successfully applied to imaging spectrometer data (Ben-Dor et al., 2009; Clark, 1999; Clark & Roush, 1984; Viscarra-Rossel et al., 2006).

Within this context, the authors have been optically characterized red dust widespread on soils by laboratory and field analyses and used hyperspectral remote sensing data to map its distribution on soils in the surrounding of the impoundment area of an aluminium plant in Montenegro.

In situ spectral analyses were carried out to characterize and separate the red dust optical spectral features and shapes from other soils and backgrounds as well as to construct a spectral library of different materials useful for calibrating and validating the remote sensing data acquired within the study area. At ground reflectance spectra in the 350–2500 nm range were acquired on red mud and red dust widespread on nearby soils using a field portable spectrometer (ASD), from a height of 1 m using a field of view of 25° and a spectralon panel in the same geometry (i.e. at-nadir on the samples) as a white reference to enable directly conversion of the measurement data into reflectance values.

Moreover, several dry red mud samples from the red mud impoundment and soil samples with different concentrations of surfacing red dust were collected for laboratory analyses.

The mineral composition of the collected samples was determined by XRD. Pure dried red mud samples and red dust polluted soils were analysed for bulk mineralogy on randomly oriented powders of whole rocks (Srodon et al., 2001). The samples were scanned from 2° to 70° 2θ. The XRD patterns of the pure red mud samples has confirmed the main presence of hematite, goethite, gibbsite and boehmite, rutile and sodium as sodium aluminium silicates or hydrosilicates, and of different red dust polluted soil samples exhibit, instead, the main presence of quartz, phyllosilicates, feldspar and carbonates, and goethite/hematite are also present.

The chemical abundance of major and trace elements (e.g., Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Ga, As, Rb, Sr, Y, Zr, Se and Pb) in the samples were measured using tube excited EDXRF. Using this system, optimum sensitivity for a particular element is achieved having the dual effect of improving sensitivity and reducing background.

Finally, MIVIS hyperspectral airborne remote sensing data collected over the study area were calibrated to radiance and atmospherically and geometrically corrected to obtain reflectances from the Visible to the Short Wave Infrared (0.4-2.5 μm) (Bassani et al., 2006).

The reflectance spectral library analysis of the collected red dust samples showed that the dominant spectral features in the VIS-NIR (0.4-1.5) range are primarily related to the iron oxides (hematite - Fe_2O_3 and/or goethite - $Fe^{3+}O(OH)$) absorption features. Phyllosilicates (clay minerals) are also largely present in the samples in the form of gibbsite and their strong spectral absorption feature in the SWIR region due to a combination of the O-H stretching fundamental with the Al-O-H bending mode (Clark, 1999) severely influences the SWIR spectral behaviour of the red dust polluted soil samples.

Figure 6 shows an example of reflectance spectra (black line) acquired by ASD spectrometer under controlled conditions in our laboratory on a representative soil sample with a high concentration of red dust on its surface in comparison with the pure USGS spectra (available at: speclab.cr.usgs.gov/spectral-lib.html) of the main minerals constituent of the red dust samples as derived from XRD analysis. This comparison was performed for the each spectrum acquired in the field in order to individuate which mineral constituent primarily influences the red dust spectrum shape and absorption features. Results attained for the analyzed polluted soil samples show that, even on naturally red soils, the red dust deposited from wind is spectrally detectable if a high level of red dust is present on it.

In Figure 7 is presented the map of the red dust distribution on soils as obtained by classifying airborne hyperspectral reflectance data using a simple and fast spectral-shape based algorithm (i.e. the Spectral Feature Fitting procedure, see Segl et al., 2003 and the references therein). The reference soil field spectra in the 0.4-2.5 μm spectral region were scaled to match the image spectra after they were normalized with the continuum removal approach to allow the comparison of individual absorption features using a common

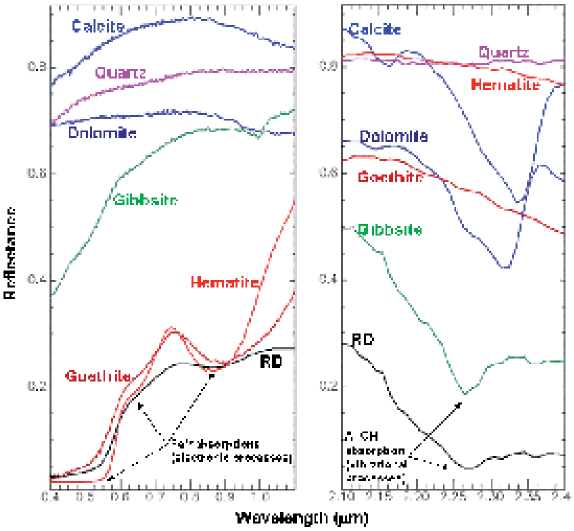

Fig. 6. Example of a lab ASD reflectance spectra (black line) acquired under controlled conditions on a typical soil sample with high level of red dust (RD) on its surface in comparison with the pure USGS spectra of the main minerals composing the RD samples.

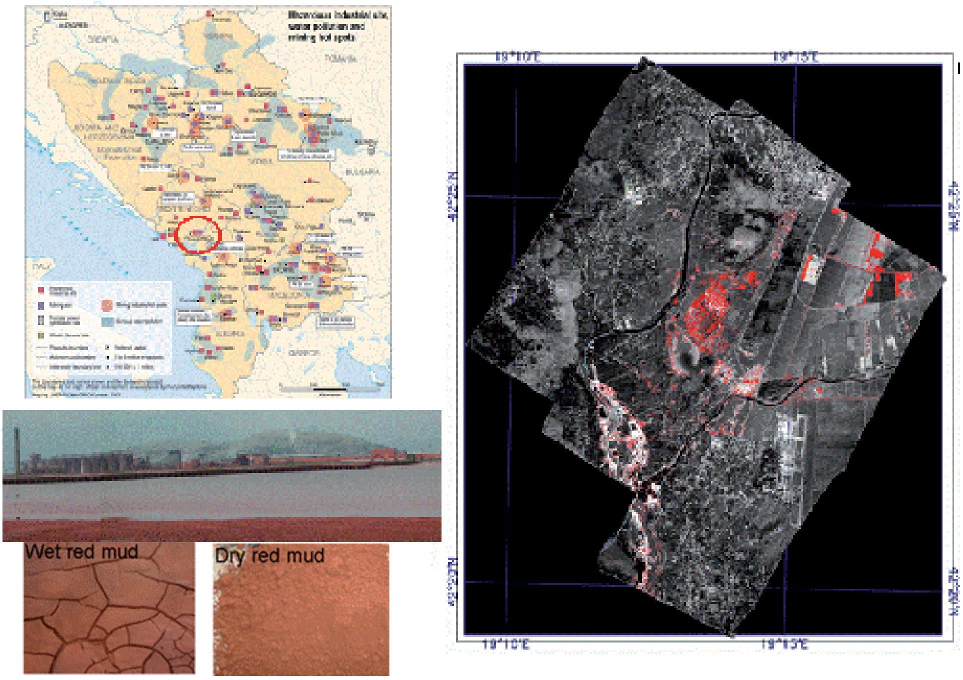

Fig. 7. Location of the study site on a map by UNEP/DEWA/GRID-Europe (2007) showing the hazardous industrial sites, water pollution and mining hot spots in eastern Europe and photos of the investigated site (on the left); red dust map depicted in red over MIVIS band 13 (0.68 µm) (on the right).

baseline. Based on visual field accuracy assessment and laboratory analysis to more quantitatively verify our results, we were able to confirm that the red dust map attained for the chosen study area has reached a good level of accuracy (i.e. an Overall Accuracy of 86%). The attained promising results highlight the usefulness of hyperspectral remote sensing data for mapping hazardous industrial pollutant, such as the red dust deposition on soils, providing their exact position.

3. Solidification/stabilization technologies for soil remediation: use of fly ash and red mud

Over the last few years a great deal of research has been carried out in order to develop remediation methods for reducing environmental risks due to polluting metal and several soil remediation technologies are based on physico-chemical processes of solidification\stabilization (S\S). In general, solidification refers to the physical encapsulation of the contaminant in a solid matrix while stabilization includes chemical reaction to reduce contaminant mobility (Mulligan et al., 2001). The S\S process could be applied both in laboratory and in situ showing good results against the risk with the remarkable benefit of immobilizing heavy metal inside natural minerals, such as clays and zeolite or soil-compatible materials. Among these, fly ash and red muds are widely used (Apak et al., 1998; Castaldi et al., 2010; Ciccu et al., 2003; Coruh & Nur Ergun, 2010; Dermatas & Meng, 2003; Garau et al., 2011; Glenister & Thornber, 1985; Gray et al., 2006; Lombi et al., 2002a; McPharlin et al., 1994; Summers et al., 1996).

In particular, many authors show that amendment of contaminated soil with red mud results in a durable reduction in metal mobility and also in a smaller risk of metal re-mobilization if soil pH were to decrease (Gray et al., 2006; Lombi et al., 2002a). Detailed experiments on the evaluation of the interaction mechanisms between red mud and heavy metals also indicate that only low toxic elements concentration absorbed by red muds are in the water-soluble and exchangeable form while the greatest concentration of metals absorbed are tightly bound and would not be expected to be released readily under natural conditions (Santona et al., 2006).

Application of red mud can also lead to a reduction in heavy metal uptake by plants (Friesl et al., 2003; Lombi et al., 2002b; Muller & Pluquet, 1998).

Other methods for reducing environmental risks lean towards toxic element immobilization using fly ash or zeolite synthesized from fly ash.

The addition of fly ash during S/S treatment of heavy metal contaminated soil is mainly responsible for their effective immobilization by absorbing the waste species on their surfaces or determining precipitation mechanisms (Dermatas & Meng, 2003; Singh & Pant, 2006; Vandecasteele et al., 2002). Precipitation of heavy metals results from the presence of calcium hydroxide, while adsorption may be due to the presence of silica and alumina available in fly ash.

Synthetic zeolite can be added to polluted soils (Querol et al., 2006; Lin et al., 1998; Rayalu et al., 2006) or crystallized directly in those contaminated (Belviso et al. 2010b; 2010c; Terzano et al., 2006) in order to solve environmental problems.

Zeolites are hydrated aluminosilicate minerals with a three-dimensional open structure making them very useful for solving the mobility of toxic elements in a number of environmental applications (Babel and Kurniawan, 2003; Ćurković et al., 1997; de'Gennaro et al., 2003; Inglezakis et al., 2002, 2003; Kesraoui-Ouki et al., 1994; Kocaoba et al., 2007;

Moreno et al., 2001a, 2001b; Ouki and Kavannagh, 1999; Pansini & Colella, 1990; Querol et al., 1999, 2001, 2002; 2006; Rayalu et al., 2006; Stefanović et al., 2007; Torracca et al., 1998; Woolard et al., 2000; Wu et al., 2008). All this is strictly connected with their ability to exchange cations, their large surface area, and their typical structural characteristics (such as porosity), which facilitate pollutant absorption and encapsulation.

This mineral can be synthesized from different source materials and fly ash is one of the most used (Berkgaut & Singer, 1996; Querol et al., 2002; Shih & Chang, 1996; Shigemoto et al., 1993). Numerous methods have been suggested for the zeolite synthesis including hydrothermal reaction (Holler & Wirsching, 1985; Murayama et al., 2002; Querol et al., 1995; 1997a; 2001; Shih and Chang, 1996; Tanaka et al., 2003), hydrothermal reaction with a fusion pre-treatment (Berkgaut & Singer, 1996; Chang & Shih, 1998; Rayalu et al., 2000; Shigemoto et al., 1993, 1994), molten-salt methods (Park et al., 2000a; 2000b), methods employing microwaves (Inada et al., 2005; Katsuki et al., 2001; Querol et al., 1997b; Slangen et al., 1997) and ultrasonic treatments (Belviso et al., 2011; Lie et al. 1995; Park et al., 2001; Wang et al., 2008). Distilled water is used in most of the experiments conducted with these different methods, whereas the synthesis of zeolite with seawater is described in very few articles (e.g. Belviso et al., 2009; 2010a; Lee et al, 2001).

The authors carried out experiments on zeolite synthesis at low temperature in a soil artificially contaminated with heavy metals (in separate experiments) and treated with fly ash. The role played by this mineral in the immobilization of heavy metals was investigated (Belviso et al. 2010b; c). The results obtained show that the direct synthesis of zeolite takes place readily after a month and the amount of the newly-formed mineral increases during the entire experimentation period. The presence of heavy metal does not exert any influence on zeolite formation which, on the contrary, plays a leading role in the mechanism of the toxic element immobilization. In fact, a reduction in toxic element availability characterizes the soil samples in which zeolite was synthesized. In particular the data about Ni (Belviso et al., 2010b) and Pb suggest that the mobilization of this elements takes place only after zeolite structure is destroyed. This causes the availability of the metals, previously trapped in the mineral and/or co-precipitated on its surfaces in the oxidable and hydroxide form respectively. In all cases synthetic zeolite forms complexes with the toxic metals which are broken by a strong chemical attack but are stable under normal environmental conditions.

4. Conclusion

Soil pollution is a worldwide environmental problem and the current technologies used for remediation are generally very expensive. In this context, the development of low cost remediation methods using various industrial residues which do not alter the physical and chemical properties of soils plays a leading role. This would also reduce waste disposal giving new value to industrial wastes through converting them into industrial by-products.

Particularly fly ash and red muds could be cost-effective materials capable of treating a variety of contaminants.

A deeply characterization of this waste materials by multi-technique approach is fundamental for their application. In particular, in this study the application of field and laboratory imaging spectroscopy for identifying and mapping soils containing pollutants, such as red dust, was successfully used in a multi-technique approach for waste material detection and soil quality and remediation strategies assessment.

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6

Heavy Metals Contaminated Soils and Phytoremediation Strategies in Taiwan

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1. Introduction

Heavy metals (HMs) in soils primarily result from the weathering of parent materials and from human activities, which including mining, smelting, application of sludges, and discharge of wastewaters, etc. (Kabata-Pendias & Pendias, 2001). Soil contaminated with HMs has become a worldwide problem and pose a serious threat to the environment (Anwar et al., 2009), leading to losses in agricultural yield and hazardous health effects as they enter the food chain (Salt et al., 1995). Cropping lands contaminated by HMs were mostly resulted from the use of polluted irrigated water in the downstream of discharged water of industrial parks of Taiwan. In 2007, approximate 400 ha of rural soils in Taiwan were contaminated with single or combined HMs and they are fallow according to Soil and Groundwater Pollution Remediation Act (SGWPR Act) announced in 2000 by Taiwan Environmental Protection Administration (Taiwan EPA). There were many techniques been used to treat the HMs-contaminated soils which including isolation, mechanical separation, chemical treatment, electrokinetics, soil washing, and phytoremediation (Mulligan et al., 2001). Various soil amendments were applied to the HMs-contaminated soils to reduce the mobility of HMs in the contaminated soils and thus to decrease their further uptake by crops (Chen & Lee, 1997; Kabata-Pendias and Pendias, 2001; Lee et al., 2004; Mench et al., 1994).

In Taiwan, most of these contaminated sites were restored with turnover/dilution and acid washing methods to reduce the total concentration of HM to conform the regulation announced. Besides the two techniques, phytoremediation was demonstrated to be a feasible method in treating these contaminated lands, which have large areas and low to medium level of HM concentration (Lai et al., 2005). It also accompanies with other environmental agenda, such as biomass energy, biodiversity, carbon sequestration, and soil quality (Dickinson et al., 2009). However, most hyperaccumulators used in removing these HMs have lower biomass and growing rate and thus extending the period needed in decontamination. The application of chemical agents has significant effect on increasing the phytoavailability and accumulation of HMs of plants (Chen & Cutright, 2001; Meers et al., 2004; Meers et al., 2005). However, results of most previous studies showed that chemical agents have negative effect on the growth of Indian mustard, sunflower, or corn and thus decreased the total removal of HMs by plants (Blaylock et al., 2007; Madrid et al., 2003; Turgut et al., 2004). After the application of chemical chelating agents, the risk of groundwater contamination may be increased because the mobility of HMs increased (Jiang et al., 2003; Lai & Chen, 2004; Lai et al., 2005). For those lands with sandy texture or high level of groundwater table, chemical agents should be carefully applied to decrease the health risk of groundwater quality (Lai & Chen, 2006; Wu et al., 2004).

Rice dominates the daily intake of cereals in most Asian countries. In Taiwan, about half of arable land is used as rice-growing field and two rice varieties including Indica and Japonica varieties are cultivated, but the latter is the major one (90%) because of taste preferences. Cadmium (Cd), normally occurs in low concentrations in soils (Wagner, 1993), is a non-essential element for plants and potentially toxic pollutant all over the world. The toxicity of Cd to plant growth, phytosynthesis, carbonhydrate metabolism, and enzyme activities is well documented (Javed & Greger, 2011; Sanita di Toppi & Gabrielli, 1999). Elevated levels of arsenic (As) in soils may potentially enter food chain (Meharg & Hartley-Whitaker, 2002) and increase the risk of cancer development (Anderson et al., 2011). According to the SGWPR Act, the cropping land with total soil Cd concentration (aqua regia soluble) exceeding 5 mg kg^{-1} will be announced as Soil Pollution Control Site (SPCS) and all farming activities are not allowed. However, many previous field surveys showed Cd-contaminated rice can still be produced from fields with total soil Cd levels lower than 5 mg kg^{-1}. The Standard for the Tolerance of Cd in rice has been reduced from 0.5 mg kg^{-1} to 0.4 mg kg^{-1} in 2007. Many studies were also subsidized by governments to assess the food safety of rice cultivated in Cd-contaminated soil. In this paper, we reviewed some previous researches regarding the accumulation of Cd and As of different rice varieties. Its safety after growing in As- or Cd-contaminated soils was also evaluated. For those contaminated lands not suitable for planting crops, the use of phytoremediation and planting non-edible plants may be a candidate for solving this problem. Experimental results of phytoremediation were also illustrated in this paper.

2. Phytoremediation for potted Cd-contaminated soils

The selection of suitable plants is the first and the critical step in conducting a successful phytoremediation. These plants should grow well and accumulate higher concentration of HMs in the harvestable parts when growing in HM-contaminated soils. There were approximately 420 species of plants that can be regarded as hyperaccumulators (Baker et al., 2000). A pot experiment was conducted to test the accumulation capacity of five garden flower species, which was regard as a potential hyperaccumulator previously (Chen & Lee, 1997). Seedlings of them were planted in the artificially Cd-contaminated loamy soils to assess their Cd accumulation when growing in control (Cd-CK) (0.43±0.15 mg kg^{-1}), Cd-10 (9.73±0.05 mg kg^{-1}), and Cd-20 (17.6±0.8 mg kg^{-1}) (Lin et al., 2010). One seedling of Star cluster (*Pantas lanceolata* Deflers.), French marigold (*Tagetes patula* L.), Impatiens (*Impatiens walleriana* Hook. f.), Garden verbena (*Verbena bipinnatifida* Nutt.), or Scarlet sage (*Salvia splendens* Ker-Gawl.) was planted in each pot contained three kilograms of artificially Cd-contaminated soils. The pot experiment was conducted in a 30/25°C (day/night) phytotron in three replicates, controlled the soil moisture content at 50-70% water-holding capacity

(WHC) by adding deionized water, and harvested after growing for 35 days. After pretreatment, the Cd concentrations in the tissues were determined.

The Cd concentrations in initial seedlings of five plants were not detectable (Cd < 0.38 mg kg^{-1}). After growing in the artificially Cd-contaminated soils for 35 days, five tested plants can significantly accumulate much higher Cd concentrations in their shoots relative to Cd-CK. Among the five plants, Impatiens grown in the Cd-20 treatment had the highest shoot Cd concentration near 100±11 mg kg^{-1}, which was more than the threshold of a Cd hyperaccumulator (100 mg kg^{-l}) reported (Baker et al., 2000). French marigold grown in Cd-10 and Cd-20 treatments accumulated 44.9±0.7 and 66.3±6.5 mg kg^{-l} in their shoots and no toxic symptoms were observed in the appearance during pot experiment. Chen & Lee (1997) reported that Star cluster, Scarlet sage, and Impatiens can accumulate 44, 12, and 42 mg kg^{-1}, respectively, in their leaves when *in-situ* growing in a Cd-contaminated site (Tatan village) in northern Taiwan. For another *in-situ* experiment carried out in Chungfu village in northern Taiwan, the final Cd concentration in their leaves was 247 mg kg^{-1} in French marigold, 52 mg kg^{-1} in Garden verbena, 12 mg kg^{-1} in Impatiens, and 11 mg kg^{-1} in Scarlet sage, respectively. French marigold and Impatiens used in this study accumulated higher Cd concentration in their shoots compared with the result of previous study possibly resulted from the higher phytoavailability of Cd in artificially Cd-contaminated soils.

Besides the accumulated concentration, bioconcentration factor (BCF = shoot HM concentration/soil HM concentration) and translocation factor (TF = shoot HM concentration/root HM concentration) were two indexes most used to evaluate the accumulating capacity of HMs by plants. For a Cd hyperaccumulator (Baker et al., 2000; Mattina et al., 2003), the BCF and TF should more than one besides the high concentration accumulated (100 mg kg^{-1}) (Sun et al., 2009). Experimental result of this study showed that the BCF values of French marigold, Impatiens, Garden verbena, and Scarlet sage were all more than one and ranged from 1.75 to 5.68 (Table 1). However, Impatiens was the only one that its TF was in the levels of 1.01-1.66. According to the standards summarized by Sun et al. (2009) for a Cd hyperaccumulator, Impatiens was a potential Cd hyperaccumulator when growing in the artificially Cd-contaminated soils. The pot experimental result was against with the in-situ selection experiment, possible resulted from the special variation and interaction of HMs in the field.

The total removal of Cd by plants determines the duration needed in decontamination. Although root of plants accumulated higher concentration of Cd in compartment with shoot, the total removal of Cd by shoot was larger because of its larger biomass (Fig. 1). Among the five tested plants, the shoots of French marigold and Impatiens removed 380-510 and 790-820 g Cd $plant^{-1}$ from Cd-10 and Cd-20. One can calculate the period needed for phytoremediation in an ideal situation, i.e. if the removal of plants of each harvest is a constant and the phytoavailability of Cd will not change with time, etc. It will take approximately 4.6-8.0 years for continuous planting (six times $year^{-1}$) French marigold and Impatiens to decrease the current Cd concentration (Cd-10 and Cd-20) to below the SPCS for cropping lands (5 mg kg^{-1}). The major drawback of phytoremediation is that it always consumes longer period compared with other techniques. Experimental results show that planting French marigold and Impatiens in Cd-contaminated soils seems to be a feasible method to remove Cd from contaminated soil and the period needed for decontamination is acceptable.

Plants	Treatments*	Shoot	Root	BCF	TF
		------------ mg kg^{-1}	------------		
Star cluster	Cd-CK	ND b	ND a	----	----
	Cd-10	8.20±0.94 a#	22.7±2.02 a	0.84	0.36
	Cd-20	10.7±2.8 a	18.9±17.2 a	0.61	0.57
French marigold	Cd-CK	ND c	ND c	----	----
	Cd-10	44.9±0.7 b	65.0±17.8 b	4.61	0.69
	Cd-20	66.3±6.5 a	113±21 a	3.77	0.59
Impatiens	Cd-CK	ND c	ND b	----	----
	Cd-10	48.9±11.7 b	29.5±9.6 ab	5.02	1.66
	Cd-20	100±11 a	99.0±8.4 a	5.68	1.01
Garden verbena	Cd-CK	ND b	ND b	----	----
	Cd-10	21.5±5.5 a	39.3±13.5 a	2.21	0.55
	Cd-20	7.63±1.75 b	49.5±11.2 a	0.43	0.15
Scarlet sage	Cd-CK	ND b	ND c	----	----
	Cd-10	21.8±7.6 a	45.9±8.2 b	2.24	0.47
	Cd-20	30.8±5.3 a	71.0±15.5 a	1.75	0.43

* Total Cd concentration digested by aqua regia: Cd-CK = 0.43±0.15 mg Cd kg^{-1}; Cd-10 = 9.73±0.05 mg Cd kg^{-1}; Cd-20 = 17.6±0.8 mg Cd kg^{-1}

\# means ± standard deviation (n = 3); ND: not detectable; The different small letters within same column of same plant tissue stand for statistical significance ($p < 0.05$)

Table 1. The Cd concentration, bioconcentration factor (BCF), and translocation factor (TF) of Star cluster, French marigold, Impatiens, Garden verbena, and Scarlet sage grown in the different Cd-treated soil for 35 days (Lin et al., 2010).

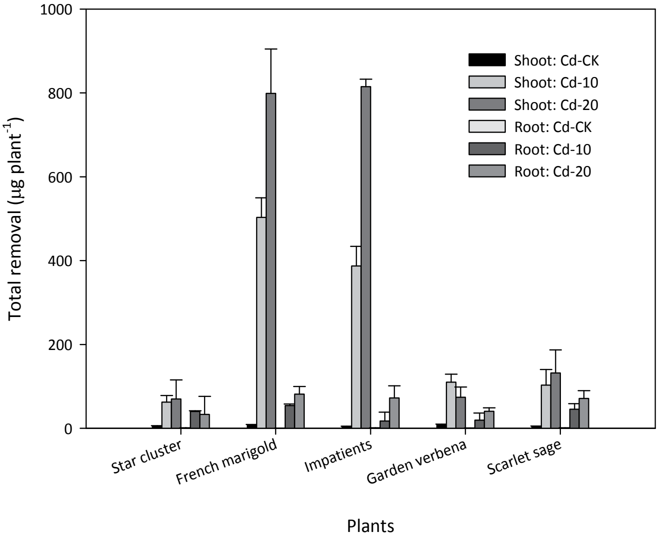

Fig. 1. The total removal of Cd concentration of Star cluster, French marigold, Impatiens, Garden verbena, and Scarlet sage grown in the different Cd-treated soil for 35 days (Lin et al., 2010).

3. Phytoremediation for Cr, Cu, Ni, and Zn-contaminated soils

Eight blocks (11 m by 4 m) located in central Taiwan (Fig. 2), which were relatively higher in Cr (chromium), Cu (copper), Ni (nickel), and Zn (zinc) concentrations were used for *in-situ* selection experiment (Lai & Chen, 2009). After making a market survey, 33 plant species of garden flower species (Table 2) were selected for this *in-situ* experiment and two seedlings per plant species were planted in each of the eight blocks. The interval space between two plants was controlled at about 50-70 cm to avoid interference. The concentration of Cu, Cr, Ni, and Zn in the 33 plant species before planting was determined. We recorded the growth condition of plants, whether toxic symptom occurred or not, two weeks after planting in the site and they were harvested after planting for 33 days. After pretreatment, the Cu, Cr, Ni, and Zn concentration in the digest solution of initial and harvested plants were determined.

After *in-situ* growing in the contaminated soils for two weeks, we observed withered and yellow color in the leaves of Bougainvillea and some of them fell off. The flowers of Cockscomb were damaged and their color changed from red (before planting) to yellow. Except for the two plants, there were no observed injures for the other 31 plants grown in the 8 blocks. Before planting, the initial concentration of HMs in the shoot were in the levels of ND (< 1.71 mg kg^{-1}) to 37.2 mg Cu kg^{-1}, ND (< 4.65 mg kg^{-1}) to 21.7 mg Cr kg^{-1}, and 8.07 to 103 mg Zn kg^{-1}. These 33 plant species have low Ni concentration (< 10.1 mg kg^{-1}) in their tissues before planting, except for Bougainvillea, Common melastoma, and Garden Canna. Although Cockscomb showed yellow flowers at second week after planting, it accumulated the highest Cu, Cr, Ni, and Zn concentration in its shoots in relative to other plant species. Cockscomb and rainbow pink accumulated 317±117 and 231±73 mg Cr kg^{-1} in their shoots, respectively, after *in-situ* growing for 33 days in the site. Their BCF was 1.7 in cockscomb and 1.3 in rainbow pink.

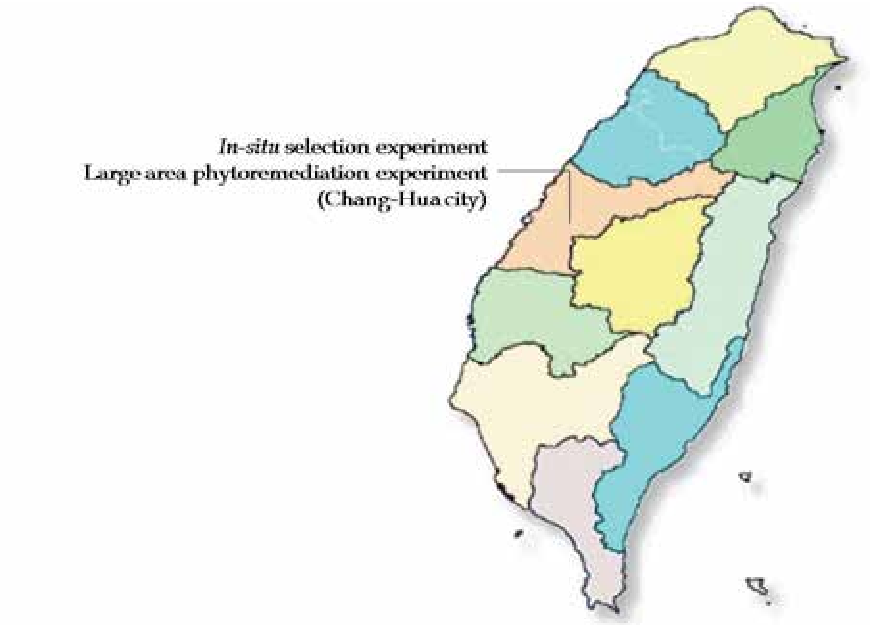

Fig. 2. Map of Taiwan and location where the phytoremediation experiments were conducted (Lai & Chen, 2009; Lai et al., 2010a).

No.	Plant species	Scientific name	Property
1	Bougainvillea	*Bougainvillea spp.*	woody
2	Rainbow pink	*Dianthus chinensis*	herbaceous
3	Serissa	*Serissa japonica*	woody
4	French marigold	*Tagetes patula*	herbaceous
5	Rose of Shoron	*Hibiscus syriacus* L.	woody
6	Water willow	*Salix warburgu*	woody
7	Chinese ixora	*Ixora chinensis* Lam.	woody
8	Sunflower	*Helianthus annuus*	herbaceous
9	Chinese hibiscus	*Hibiscus rosa-sinensis*	woody
10	Gold dewdrop	*Duranta repens*	woody
11	Kalanchoe	*Kalanchoe blossfeldiana*	herbaceous
12	Creeping Trilobata	*Wedelia trilobata*	herbaceous
13	Garden Canna	*Canna generalis spp.*	herbaceous
14	Garden verbena	*Verbena hybrida*	herbaceous
15	Malabar chestnut	*Pachira macrocarpa Walp*	woody
16	Purslane	*Portulaca oloraua* Linn	herbaceous
17	Common Lantana	*Lantana camara* L.	woody
18	Fancy leaf caladium	*Caladium hortulanun* Birdsey	herbaceous
19	Coleus	*Coleus blumei*	herbaceous
20	Golden trumpet	*Allamanda cathartica* Linn	woody
21	Common melastoma	*Melastoma candidum*	woody
22	Carland flower	*Hedychium coronarium*	herbaceous
23	Manaca raintree	*Brunfelsia uniflora*	woody
24	Yellow Cosmos	*Cosmos sulphureus*	herbaceous
25	Sliver apricot	*Ginkgo biloba* L.	woody
26	Temple tree	*Plumeria acutifolia Poir.*	herbaceous
27	Orchid tree	*Aglaia odorata Lour.*	woody
28	Star cluster	*Pentas lanceolata*	herbaceous
29	Blue daza	*Evolvulus nuttallianus*	herbaceous
30	Cockscomb	*Celosia cristata*	herbaceous
31	Scandent Schefflera	*Schefflera arboricola Hayata.*	woody
32	Bojers spurge	*Euphorbia splendens*	woody
33	Croton	*Codialum variegatum.*	woody

Table 2. The 33 plant species planted in the central Taiwan used for *in-situ* selection experiment.

For Cu, the accumulation capacity of various tested plants was in the order of Cockscomb (117±40 mg kg^{-1}), Garden verbena (84.7±46.6 mg kg^{-1}), and Star cluster (80.4±80.6 mg kg^{-1}). The average Cu concentration of corn and food grains of China was 2.67 and 6.46 mg kg^{-1} (Chen et al., 1994) and the Cu concentration for foodstuff crops was less than 10 mg kg^{-1} (Kabata-Pendias & Pendias, 2001). The Cu concentration in the brown rice of Japan and Indonesia was 2.16-4.4, 2.9, and 3.41 mg kg^{-1}, respectively (Iimura, 1981; Masironi, 1977; Suzuki et al., 1980). Although the accumulated Cu concentration of these 33 plants increased after 33 days, the BCF were less than 1.1 because the surface soil has low Cu concentration, ranged from 112 to 122 mg kg^{-1}. Because of the low Ni concentration in the initial plants, the Ni concentration of shoot in the 33 plants increased after *in-situ* planting in the contaminated site for 33 days. The Ni concentration of shoot was in the order of Cockscomb (145±38 mg kg^{-1}), French marigold (90.9±42.4 mg kg^{-1}), and Garden verbena (88.0±36.8 mg kg^{-1}). Because of the high Ni concentration in the surface soil of the 8 blocks (ranged from 314 to 412 mg kg^{-1}), the accumulated Ni concentration in the shoots of plants increased after *in-situ* planting for 33 days. After 33 days, the accumulated Zn concentration in the shoots of plants was in the order of Cockscomb (435±127 mg kg^{-1}), Garden verbena (339±210 mg kg^{-1}), and Yellow Cosmos (328±157 mg kg^{-1}). However, the BCF of Zn of Cockscomb was only 0.7 which revealed that the accumulation capacity of these 33 plants was weak.

Experimental results also show that the accumulation of HMs of woody and herbaceous plants after growing for 33 days was quite different (Table 2). Similar to the results of Chen & Lee (1997), herbaceous plants have accumulated higher concentration of HMs in comparison to woody plants. Except for the low Ni concentration in initial plants, the increase for HMs concentration in woody plants was about 3.1±2.9 fold for Cu, 2.5±1.5 fold for Cr, and 4.3±3.1 fold for Zn, respectively. Herbaceous plants have higher uptake of HMs in relative to woody plants and their increase on the concentration of HMs are 9.4±6.5 fold for Cu, 5.1±2.7 fold for Cr, and 8.9±6.1 fold for Zn.

4. Large area phytoremediation experiments of 12 plant species in HMs-contaminated site

Twelve plants species (Table 3) were selected from 33 plant species testing in a site contaminated by combined HMs in central Taiwan (Fig. 2) to study the feasibility of *in-situ* phytoremediation (Lai et al., 2010a). Soil samples of topsoil (0-15 cm) and subsoil (15-30 cm) were collected and analyzed for the total concentration of eight HMs (As, Cd, Cr, Cu, Hg, Pb, Ni, and Zn). The studied site was mainly contaminated by Cr, Cu, Ni, and Zn and some of the concentrations were much higher than the SPMS (Soil Pollution Monitoring Standard) or SPCS. The total area for each plant species was approximately 0.1 ha and their planting density was 10,000 seedlings ha^{-1}. Plants were harvested after growing for one and two months and their concentration of Cu, Cr, Ni, and Zn in the shoot were determined.

Results of two times of large area experiments after foregoing 12 plant species were growing for one month and two months showed that they can grow well in this combined HMs-contaminated site. The concentrations of Cr, Cu, Ni, and Zn in the shoots increased after growing for 31 days compared with those of it before planting. The extension of their time of growth, from one month to two months in the contaminated site, has positive effects on increasing their accumulation of HMs. However, the 12 tested plant species could not accumulated higher concentrations of Cr, Cu, Ni, and Zn possibly resulted from the lower concentrations of foregoing HMs. None of the plant species can regard as a hyperaccumulator according to the definition of Baker et al. (2000). After *in-situ* growing in the contaminated situ for one month, the roots of most of the 12 plant species accumulated higher concentrations of HMs compared with the shoots. Among the four HMs, the TF of Zn was highest in comparison to others. The TF of some tested plant species were more than one from the result of *in-situ* experiment (Table 4). In an ideal situation, it will take about 4.3 to 66 years by planting foregoing plant species to reduce the concentrations of HMs to conform the SPMSs.

No.	Plant species	Scientific name	Property
1	Chinese ixora	*Ixora chinensis* Lam.	woody
2	Garden verbena	*Verbena hybrida*	herbaceous
3	Rainbow pink	*Dianthus chinensis*	herbaceous
4	Bojers spurge	*Euphorbia splendens*	woody
7	Kalanchoe	*Kalanchoe blossfeldiana*	herbaceous
5	Scandent Scheffera	*Schefflera arboricola* Hayata.	woody
6	Purslane	*Portulaca oloraua* Linn	herbaceous
7	Croton	*Codialum variegatum.*	woody
8	Serissa	*Serissa japonica*	woody
9	Garden Canna	*Canna generalis spp.*	herbaceous
11	French marigold	*Tagetes patula*	herbaceous
12	Sunflower	*Helianthus annuus*	herbaceous

Table 3. The 12 plant species planted in the central Taiwan used for large area phytoremediation experiment.

Plant species	Translocation factor (TF)#			
	Cr	Cu	Ni	Zn
Chinese ixora	0.41	1.28	0.22	1.40
Garden verbena	0.35	0.64	0.33	2.13
Rainbow pink	0.58	0.78	0.89	1.17
Bojers spurge	0.84	1.44	0.75	1.98
Kalanchoe	0.15	1.31	0.28	--
Scandent Scheffera	0.07	0.84	0.61	--
Purslane	5.06	0.84	0.68	0.72
Croton	0.05	0.38	0.25	--
Serissa	0.40	0.90	0.57	--
Garden Canna	0.61	0.54	0.43	1.35
French marigold	0.18	0.56	0.30	--
Sunflower	0.21	1.39	0.41	3.15

TF = The ratio of shoot HM concentration to root HM concentration

Table 4. The translocation factor (TF) of 12 plant species after *in-situ* growing in a combined HMs-contaminated site for one month.

The median and maximum concentrations of Cr, Cu, Ni, and Zn in the topsoil were used in this study to calculate the mean and maximum effect of contaminants. The exposure risk was resulting from the ingestion of contaminated soils (EXP_{ing}), inhalation of air containing contaminated soil particles (EXP_{inh}), and absorption by skin (EXP_{abs}). Different equations were used to calculate the carcinogenic and non-carcinogenic risks of contaminants to the health of humans (Lai et al., 2011). Where HQ is the hazard quotient and EXP_{total} is the sum of total exposure. There are carcinogenic and non-carcinogenic risks when the values of HQ and TR are less than unitary and 10^{-6}, respectively. The concentrations of Cr, Cu, Ni, and Zn in the topsoil after phytoremediation were estimated by considering the removal of plants. The results showed that although the study site was contaminated with combined HMs, there are no carcinogenic and non-carcinogenic risks (Tables 5 and 6) although some of the total concentrations of Cr, Cu, Ni, and Zn were higher than the SPMSs or SPCSs.

	Zn		Cr		Cu		Ni	
	Med.	Max.	Med.	Max.	Med.	Max.	Med.	Max.
	Before phytoremediation							
EXP_{inh}	5.9×10^{-6}	1.1×10^{-5}	1.4×10^{-6}	3.5×10^{-6}	1.5×10^{-6}	2.1×10^{-6}	3.7×10^{-6}	7.0×10^{-6}
EXP_{ing}	2.7×10^{-4}	5.2×10^{-4}	6.5×10^{-5}	1.6×10^{-4}	7.1×10^{-5}	9.6×10^{-5}	1.7×10^{-4}	3.2×10^{-4}
EXP_{abs}	4.7×10^{-5}	8.9×10^{-5}	1.1×10^{-5}	2.8×10^{-5}	1.2×10^{-5}	1.6×10^{-5}	3.0×10^{-5}	5.6×10^{-5}
HQ	1.0×10^{-3}	2.0×10^{-3}	2.5×10^{-2}	6.2×10^{-2}	3.0×10^{-3}	4.1×10^{-3}	9.8×10^{-3}	1.9×10^{-2}
	After phytoremediation							
EXP_{inh}	5.8×10^{-6}	1.1×10^{-6}	1.4×10^{-6}	3.5×10^{-6}	1.5×10^{-6}	2.0×10^{-6}	3.7×10^{-6}	7.0×10^{-6}
EXP_{ing}	2.6×10^{-4}	5.1×10^{-4}	6.4×10^{-5}	1.6×10^{-4}	7.0×10^{-5}	9.5×10^{-5}	1.7×10^{-4}	3.2×10^{-4}
EXP_{abs}	4.5×10^{-5}	8.8×10^{-5}	1.1×10^{-5}	2.8×10^{-5}	1.2×10^{-5}	1.6×10^{-5}	2.9×10^{-5}	5.5×10^{-5}
HQ	1.0×10^{-3}	1.9×10^{-3}	2.4×10^{-2}	6.1×10^{-2}	2.9×10^{-3}	4.0×10^{-3}	9.7×10^{-3}	1.8×10^{-2}

Med.: median value; Max.: maximum value

Table 5. The hazard quotient (HQ) and the exposure risk of the three pathways before and after phytoremediation.

	Cr		Ni	
	Med.	Max.	Med.	Max.
	Before phytoremediation			
EXP_{inh} (mg kg^{-1} day^{-1})	2.1×10^{-8}	5.1×10^{-8}	5.3×10^{-8}	1.0×10^{-7}
TR	7.0×10^{-7}	1.8×10^{-6}	3.7×10^{-8}	7.0×10^{-8}
	After phytoremediation			
EXP_{inh} (mg kg^{-1} day^{-1})	2.0×10^{-8}	5.0×10^{-8}	5.3×10^{-8}	1.0×10^{-7}
TR	6.9×10^{-7}	1.7×10^{-6}	3.7×10^{-8}	7.0×10^{-8}

Med.: median value; Max.: maximum value

Table 6. The exposure and carcinogenic risk before and after phytoremediation if only the inhalation was considered.

5. Uptake characteristics of different rice varieties growing in Cd-contaminated soils

In 2005 and 2006, field studies were conducted in Taiwan to investigate the uptake characteristics of rice varieties growing in 19 different paddy fields in three counties across the western plains in Taiwan (Römkens et al., 2009). The studied fields were located at the towns of Chang-hua (three fields), Ho-Mei (three fields), Lu-Kang (two fields), Hsin-Chu (three fields), and Pa-Deh (eight fields) (Fig. 3). Twelve rice cultivars of Indica or Japonica varieties were planted in each field with 5-9 replicates for each cultivar depends on the field size. Samples of topsoil (0-25 cm) and rice plants at full maturity were collected together at the same location in studied fields in May (harvest 1) and November (harvest 2) of the two years. Total numbers of soil and rice plant samples in this study were both 3,198. The total soil Cd concentration in studied fields ranged from 0.06 mg kg^{-1} to as high as 27.8 mg kg^{-1}, the maximum level is about 6-fold higher than the SPCS (5 mg kg^{-1}) enacted in Taiwan. Around 27% of the studied field area was defined as Cd-contaminated soil according to the SGWPR Act.

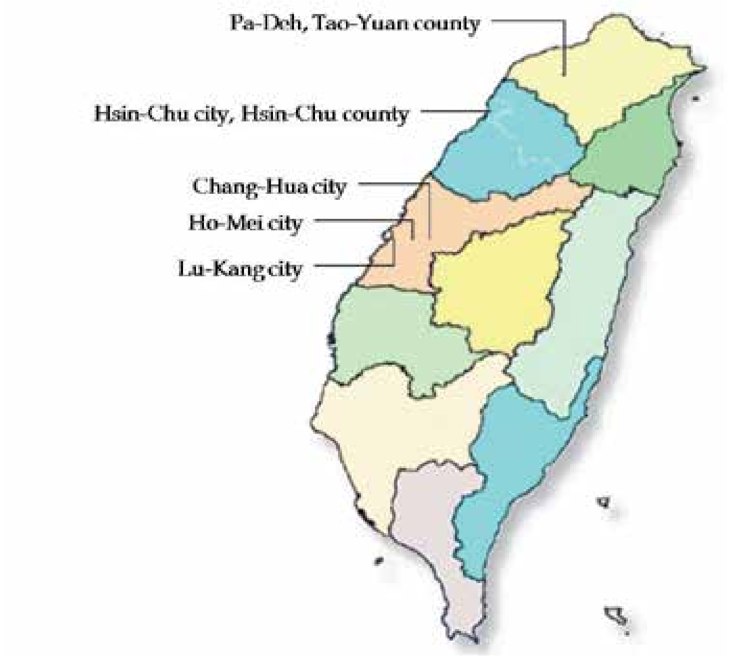

Fig. 3. Map of Taiwan and location of communities where the paddy fields are located (Römkens et al., 2009).

Soil pH, CEC (Cation Exchange Capacity), and soil organic matter (SOM) varied widely in the 19 paddy fields (Table 7). Cadmium concentrations in rice grains were quite different among cultivars even though they were planted in soils with comparable soil properties and total soil Cd levels. Overall, median Cd concentrations in rice grains of Indica variety were 2-3 times higher than that of Japonica variety no matter the rice is planted in low or high Cd-contaminated fields or in different climates (Fig. 4). Higher variation was found in the concentration of Cd in Indica brown rice compared with that in Japonica brown rice. Some studies also found that Cd accumulation in brown rice of Indica was 1.54 times higher than that of Japonica. This uptake characteristic of rice varieties is important for selecting rice cultivars with low Cd accumulating ability in rice grain planted in slightly Cd-contaminated soil.

	pH	CEC (cmol$_+$ kg^{-1})	SOM (%)	Total Cd (mg kg^{-1})
Japonica	3.8-7.2	2.6-24.2	1.4-9.5	0.06-27.8
Indica	4.1-7.0	2.6-25.1	1.3-10.2	0.08-25.9

Table 7. The pH, CEC, and SOM of the soils growing for two rice varieties.

Liu et al. (2007) reported that Cd was not evenly distributed in different parts of rice grain. The results of their pot experiments planting six rice cultivars (include Indica, Japonica, hybrid Indica, and New Plant type) in artificially Cd-contaminated soil showed that the average percentage of Cd quantity accumulated in chaff, cortex (embryo), and polished rice were about 15%, 40%, and 45%, respectively. The cortex occupied only 9% of the grain dry weight in average but the polished rice occupied 71%, so Cd concentration in cortex is significantly higher than that in polished rice. They suggested that polished rice produced from Cd-contaminated fields may be safer for consumers than brown rice. However, Moriyama et al. (2003) reported that Cd concentration in six Japonica rice cultivars reduced only 3% in average after milling process. A study using *in-situ* synchrotron X-ray fluorescence to identify Cd distribution in brown rice produced from Bangladesh, China, and U.S. also showed that Cd is evenly distributed in brown rice (Meharg et al., 2008). The inconsistent findings among these studies may be caused by errors from rice polishing process or inherent differences of Cd distribution in rice grain among rice cultivars. More careful studies are required to clarify the inconsistent results.

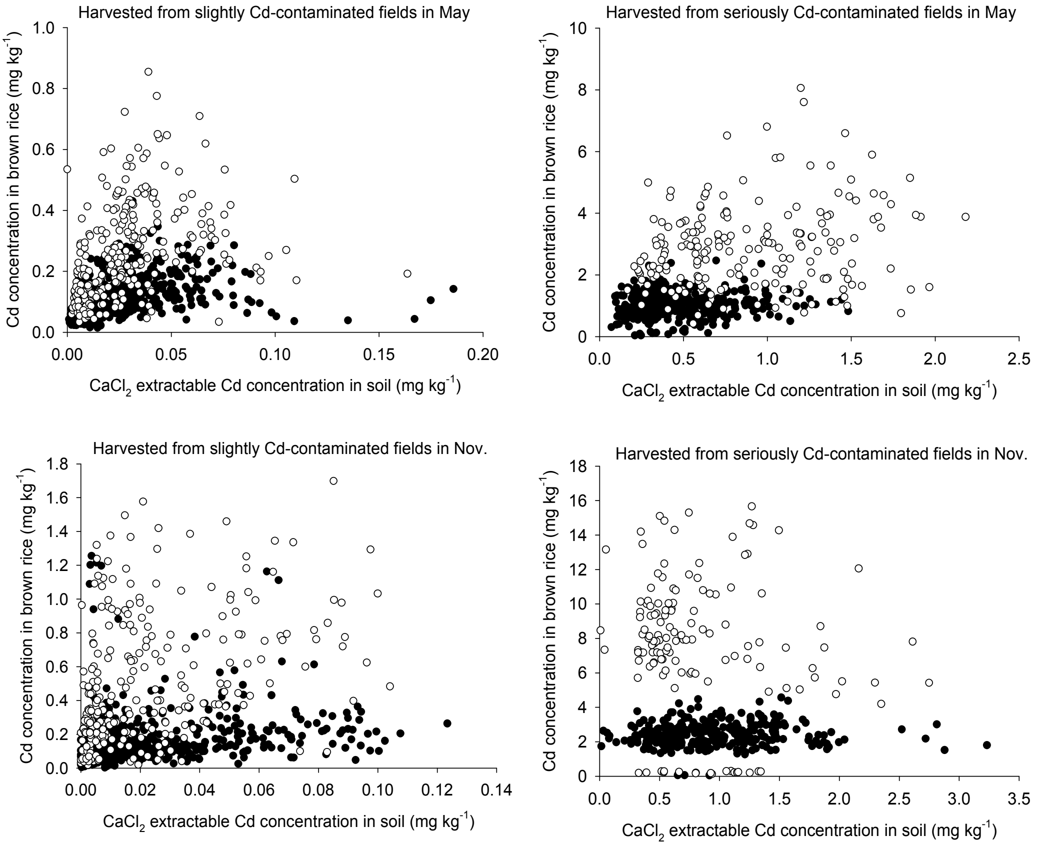

Fig. 4. The relationship between $CaCl_2$ extractable Cd concentration in soil and Cd concentration in brown rice (•: Japonica species, ◦ : Indica species) harvested in May and November.

6. Various Cd uptake models were used to efficiently predict their accumulation

Total Cd concentration in soil is not a reliable index to determine whether rice grain is safe for consumers. Rice varieties and soil characteristics such as soil pH, Eh (redox potential), CEC, texture, and SOM are important factors affecting Cd concentration in rice grain. To determine whether a rice-growing field can produce safe rice grains with Cd levels lower than FQS (food quality standard), it is necessary to develop a simple and reliable soil tests to predict available Cd concentration in rice grains.

Previous studies indicated that 0.01M $CaCl_2$, 0.1M HCl, 0.43M HNO_3, and 0.05M EDTA (Na_2-EDTA $\cdot 2H_2O$) are ideal extractants to estimate soil available Cd concentration (Houba et al., 1997; Nelson et al., 1959; Tiwari & Kumar, 1982). This study compared these methods to assess which method is better for predicting Cd levels in rice grains. The best well-performed regression equation to predict Cd levels in rice grain was presented here using soil available Cd and Zn concentrations determined by 0.01M $CaCl_2$:

$$\log[\text{Cd–grain}] = 0.94 + 0.78\times\log[\text{Cd–CaCl}_2] - 0.30\times\log[\text{Zn–CaCl}_2],\ r^2 = 0.73 \ldots \text{for Indica} \quad (1)$$

$$\log[\text{Cd–grain}] = 0.60 + 0.82\times\log[\text{Cd–CaCl}_2] - 0.28\times\log[\text{Zn–CaCl}_2],\ r^2 = 0.86 \ldots \text{for Japonica} \quad (2)$$

The $CaCl_2$ extractable Zn is also included in the equation because it is able to compete with Cd for plant uptake and reduce toxic effects of Cd. The critical concentrations of $CaCl_2$-extractable Cd in soil under different levels of soil $CaCl_2$-extractable Zn are constructed for farmers and authorities in Taiwan to prevent the production of Cd-contaminated rice by using above equations.

The concentration of $CaCl_2$-extractable Zn in soil ranged usually from 0.1 to 50 mg kg^{-1} when the total soil Zn concentration is less than 600 mg kg^{-1}, the SPCS for cropping lands enacted in Taiwan. According to the equations, less Cd will be accumulated in rice grain if the soil $CaCl_2$-extractable Zn is getting higher, therefore, only the critical concentrations of $CaCl_2$-extractable Cd in soil under the soil $CaCl_2$-extractable Zn lower than 50 mg kg^{-1} are presented. If the measured soil $CaCl_2$-extractable Cd is higher than the critical value, it is possible to produce rice grain with Cd concentration exceeding the Standard for the Tolerance of Cd in rice (0.4 mg kg^{-1}) (Table 8). Further studies are required to validate the practicability of regression equations.

Rice variety	CaCl$_2$-extractable Zn in soil (mg kg^{-1})							
Indica	0.007	0.019	0.035	0.046	0.060	0.071	0.079	0.086
Japonica	0.027	0.060	0.105	0.133	0.168	0.193	0.213	0.230

Table 8. Critical concentrations of $CaCl_2$-extractable Cd (mg kg^{-1}) in soil under different levels of soil $CaCl_2$-extractable Zn (mg kg^{-1}) for the two rice varieties. Cadmium concentration in rice grain will exceed 0.4 mg kg^{-1} if the measured soil $CaCl_2$-extractable Cd is higher than the critical concentration.

To predict Cd concentration in rice grain, Simmons et al. (2008) also developed a regression equation using soil pH (1:5) and $CaCl_2$ extractable Cd determined on field-moist samples collected during the grain-filling period. The equation can predict Cd concentrations in unpolished rice grain with an r^2 value of 0.638. If air-dried soil samples were used for Cd–$CaCl_2$ and pH determination, the regression equation cannot explain the variability of Cd levels in rice grain. Air-drying may affect soil sample conditions to an extent that $CaCl_2$ extractable Cd cannot represent Cd availability in soil compared to extracts collected from field-moist soil. However, the soil samples used for developing regression equations in the study of Taiwan as mentioned above were air-dried and collected during rice harvest period, an easier pretreatment for soil samples and more suitable for routine monitoring.

Brus et al. (2009) recently developed a multiple regression model using 0.43M HNO_3 extractable Cd, pH, clay, and SOM as predictors to predict Cd levels in rice grain harvested form the paddy fields in Fuyang, Zhejiang province, China. The model performed much better ($r^2_{adj} = 0.661$) than the linear model using only 0.01M $CaCl_2$ extractable Cd as a predictor ($r^2_{adj} = 0.281$). The field study in Taiwan as mentioned above also developed a multiple regression model using 0.43M HNO_3 extractable Cd, pH, and CEC to predict Cd levels in rice grain. Although the model using more predictors to reflect the effects of pH and CEC on the availability of Cd, it did not perform much better ($r^2 = 0.81$ and 0.74 for Japonica and Indica, respectively) than the model using 0.01M $CaCl_2$ extractable Cd and Zn as predictors ($r^2 = 0.86$ and 0.73 for Japonica and Indica, respectively). Therefore, the latter simpler model is preferred to be validated and used in Taiwan. Since different environmental and soil factors affect the accumulation of Cd in rice grain in different ways and extents, the predicting models developed by using local data will be more reliable to be used for the specific area.

7. As-contaminated soils in Guandu plain

Arsenic is a contaminant of public concern since it is highly toxic and carcinogenic. It may be accumulated in plants and eventually be transferred to humans through the food chain. A regular monitoring for HM concentrations in soil conducted by Taipei government found that some soil samples in Guandu Plain were contaminated by As. Further comprehensive survey conducted in 2006 showed that more than 60 ha of rice-growing soils located in that area were contaminated by As. The maximum As concentration in topsoil (0-15 cm) reached 535 mg kg^{-1} in this area, which was almost 9 times of the SPCS (60 mg kg^{-1}) enacted in Taiwan. The contamination source of As in this area may come from the hot spring water of Thermal Valley. The hot spring water flowed out and mixed with the stream water which was used as irrigation water for the As-contaminated area of the Guandu Plain (Chang et al., 2007). Some studies indicated that the soil parent materials may also contribute to the high levels of As in soils of Guandu Plain (Su & Chen, 2008; Wu, 2007).

Arsenic in soils occurs mainly as inorganic species (Huang, 1994). In well-aerated soils, arsenate (As(V)) is the predominate form, whereas in reduced environment such as paddy soils, arsenite (As(III)) species prevails. Previous studies showed that As(V) in aerated soils will be reduced to more mobile and toxic As(III) in paddy soils and transferred to rice (Huang, 1994; Masscheleyn et al., 1991). Since As(III) is much more toxic, more soluble, and more mobile than As(V), it is a big chance that arsenic in rice-growing soils in Guandu Plain may transfer to rice and reduce rice yield or even impairs food safety. Meharg & Rahman (2003) indicated that As levels of paddy soils in Bangladesh irrigated with As-contaminated groundwater reached only 46 mg kg^{-1} but the As concentration in rice grains were as high as 1.7 mg kg^{-1} DW. Liao et al. (2005) also reported high levels of As in rice (0.5-7.5 mg kg^{-1} DW) grown on As-contaminated soils in China. Whether the rice produced in highly As-contaminated soil in Guandu Plain is safe for human consumption or not is an emergent and important issue of local residents and government agency.

8. Uptake characteristics of two rice varieties growing in highly As-contaminated soils

In 2007, thirteen topsoil (0-15 cm) and rice (*Oryza sativa* L.) samples were collected together in 13 paddy fields (Fig. 5) with various levels of total As, ranging from 12 to 535 mg kg^{-1}, in soil according to previous survey. Two Japonica rice cultivars, Taikeng No. 8 and Tainan No. 11, were planted in the 13 paddy fields. The 13 collected soil samples were acidic (pH 4.6-5.9) and fine textured (clay content 38-58%).

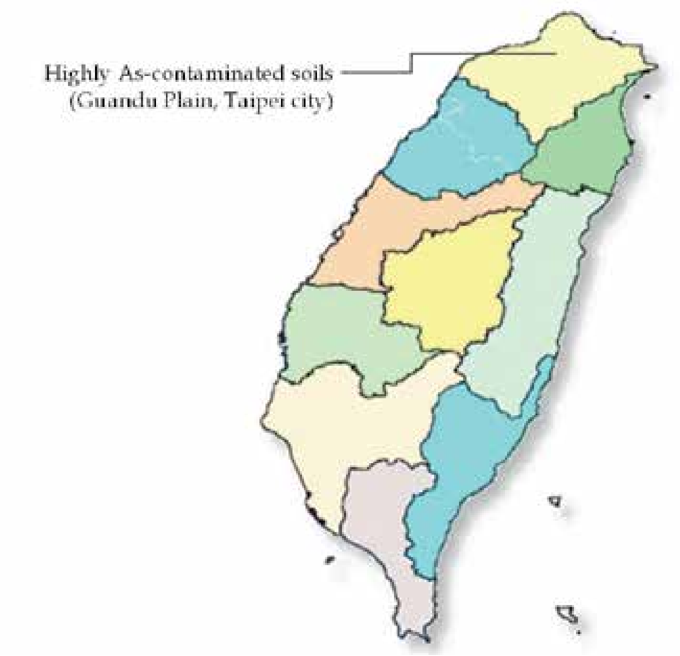

Fig. 5. Map of Taiwan and location where As-contaminated soil samples were collected (Su & Chen, 2008).

Although total soil As concentrations varied widely from 12.4 to 535 mg kg^{-1}, As concentrations in brown rice were all below 0.35 mg kg^{-1} DW and no adverse effects were shown on rice growth (Fig. 6). The Standards for the Tolerance of HMs in rice enacted in Taiwan does not include As. According to the statutory limits of As concentration in cereals or food crops constructed in different countries, the rice harvested from the As-contaminated soils in Guandu Plain was still safe for consumers.

Zavala & Duxbury (2007) suggested a global "normal" range of As concentration in rice as 0.08-0.20 mg kg^{-1}, according to the combination of data set (n = 411) from their study and literatures. They also found that As levels in rice produced from Asia were significantly lower than that from U.S. or EU (Table 9). The As concentration in the majority of rice samples from Asia were lower than 0.098 mg kg^{-1}. Compared with their findings, the As levels in rice grain produced in Guandu Plain were higher than the suggested global normal range even though they did not exceed the statutory limits. However, a pot experiment conducted in Taiwan also showed that As concentrations in brown rice ranged from 0.1 mg kg^{-1} to as high as 0.4 mg kg^{-1}, even the rice was cultivated in soils not seriously contaminated by As (total As < 25 mg kg^{-1}) (Simmons et al., 2008). In the study of Zavala & Duxbury (2007), the rice samples collected from many countries may not be representative of major rice consumption in those countries, it is necessary to conduct a comprehensive survey for As concentrations in different rice cultivars produced in Taiwan to estimate the normal levels of As in rice and compared with the data from Guandu Plain.

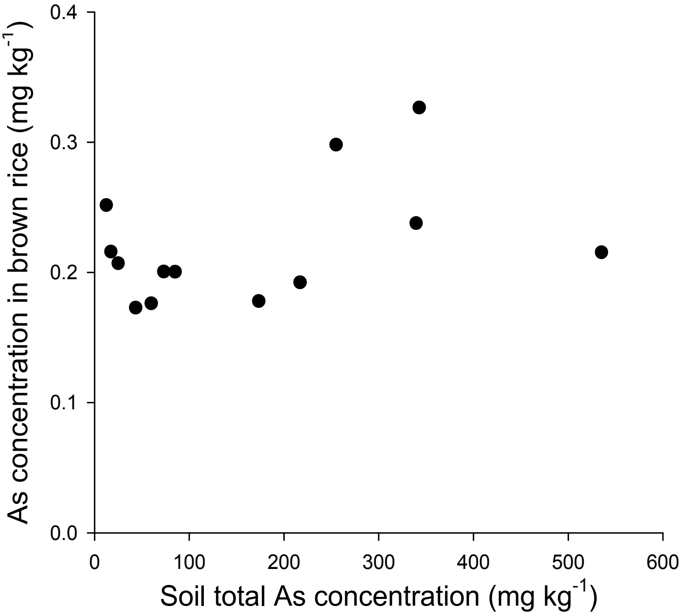

Fig. 6. The relationship between As concentration in soil and in brown rice collected together from 13 paddy fields in Guandu-Plain, north Taiwan.

Country/Institute	Regulation item	Statutory limit	Reference
Australia	cereals	1 mg kg^{-1} FW	Brus et al., 2009
Canada	food crops	1 mg kg^{-1} FW	Zandstra & Kryger, 2007
China	rice	0.15 mg kg^{-1} DW*	URL, 2005
New Zealand	cereals	1 mg kg^{-1} FW	Brus et al., 2009
Switzerland	food crops	4 mg kg^{-1} DW	Gulz et al., 2005
United Kingdom	food in sale	1 mg kg^{-1} FW	Warren et al., 2003

FW: fresh weight; DW: dry weight; *: limit for inorganic As

Table 9. The statutory limits of arsenic concentration in cereals or food corps announced by WHO or different countries.

Many studies found that the arsenic concentration in rice grain harvested from As-contaminated soil could reach above 0.7 mg kg^{-1}. However, rice produced in Guandu Plain is not apparently affected by As-contaminated soil. The availability of As in soil may be very low. To investigate the distribution of As forms associated with soil solid phases, an As-specific sequential extraction procedure proposed by Wenzel et al. (2001) was conducted for the collected 13 soil samples.

The results showed that relative portions of all As fractions were similar in 13 collected soil samples even the total soil As levels varied widely. The level of non-specifically-bound As in soil samples were all below 0.7% of total arsenic concentration in soils. Since the non-specifically-bound As represented the bioavailable As in soils and correlated well with As concentrations in soil solution collected in fields, the extremely low concentration of this As fraction may explain the facts that arsenic concentration in brown rice cultivated in highly As-contaminated soils of Guandu Plain were all below 0.35 mg kg^{-1} (Fig. 6) and no adverse effects on rice growth.

Abedin et al. (2002) conducted a pot experiment using As-contaminated irrigation water to grow rice and suggested that As can be readily transferred from root to shoot if As levels in root exceeded the As storage capacity. However, a possible protection mechanism may exist in rice straw and husk to inhibit As accumulation in rice grain because the ratio of As concentration in grain/husk/straw is around 1/10/100 at the highest arsenate treatment (As levels in irrigation water = 8 mg L^{-1}). This suggested that the suppression of As transfer from rice husk to grain may play a key role in reducing As concentration in rice grain. Since the primary As forms in soil environments are As(III) and As(V), As uptake by rice in paddy fields may mostly accumulate in rice roots. These transferring characteristics of As in rice may also contribute to the fact that low As levels in rice grain was found in Guandu Plain.

The amorphous hydrous Fe and Al oxide-bound As was the major fraction in soils (>50% of total As). This suggested that the amorphous materials in soils may play a central role in limiting the availability of arsenic in soils. However, the levels of specifically-bound As were around 10% of total arsenic in soils. Since the total arsenic concentration in some soils were very high and the application of lime materials or phosphorus fertilizer may potentially mobilize the specifically-bound As, further studies on these potential risks to agroecosystems were absolutely required.

Each fraction of arsenic in soil had significant linear relationship with total arsenic concentration in soil. This suggested that a single source of As contamination in Guandu Plain and the soil properties affected As adsorption in this area were similar. A significant linear relationship was found between $Al_o + 1/2Fe_o$ (%) in soil and total soil As concentration (mg kg^{-1}) (r^2= 0.89, $P < 0.001$). This result indicated the source of As contamination may rich in amorphous Fe and Al. Since the $Al_o + 1/2Fe_o$ (%) was good indicator of andic soil properties (Soil Survey Staff, 2006) and andesite is the parent material of soils in Guandu Plain, this findings suggested that the parent material may also contribute to the high levels of As in soils of Guandu Plain.

9. Conclusion

According to pot experiments and *in-situ* field scale experimental results, many plants can accumulate high concentration of HMs in their tissues and phytoremediation is feasible in removing HMs using suitable plant species. The critical concentrations of $CaCl_2$-extractable Cd in soil under different levels of soil $CaCl_2$-extractable Zn are constructed for farmers and authorities in Taiwan to prevent the production of Cd-contaminated rice by using the two equations developed in this study for Indica and Japonica rice cultivars. A single source of As contamination in Guandu Plain and a significant linear relationship was found between $Al_o + 1/2Fe_o$ content in soil and total soil As concentration. Although total soil As concentrations varied widely from 12.4 to 535 mg kg^{-1}, As concentrations in brown rice were all below 0.35 mg kg^{-1} (DW) even the As regulation for rice was not announced in Taiwan.

According to the statutory limits of As concentration in cereals or food crops constructed in different countries, the rice harvested from the As-contaminated soils in Guandu Plain was still safe for consumers.

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7

Biological Remediation of Hydrocarbon and Heavy Metals Contaminated Soil

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1. Introduction

Contamination of soil environment by hydrocarbons (mostly petroleum hydrocarbons) is becoming prevalent across the globe. This is probably due to heavy dependence on petroleum as a major source of energy throughout the world, rapid industrialization, population growth and complete disregard for the environmental health. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year (Kvenvolden and Cooper (2003). Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution (Holliger et al., 1997). These hydrocarbon pollutants usually caused disruptions of natural equilibrium between the living species and their natural environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants (Das and chandran, 2010).

Heavy metals contaminated soil from industrial waste; electronic wastes etc. on the other hand pose a serious threat to both man and animals in the environment if not properly remediated to the innocuous level. Environmental pollution by heavy metals which are released into the environment through various anthropogenic activities such as mining, energy and fuel production, electroplating, wastewater sludge treatment and agriculture is one of the world's major environmental problem. Heavy metals or trace metals refer to a large group of trace elements which are both industrially and biologically important. Initially, heavy metals are naturally present in soils as natural components but as of now, the presence of heavy metals in the environment has accelerated due to human activities. This is a widespread problem around the world where excessive concentration of heavy metals such as Pb, Zn, Cr, Cu, Cd, Hg, and As can be found in soils.

Soil contamination by heavy metals is consequently the most critical environmental problems as it poses significant impacts to the human health as well as the ecosystems. The contaminants are able to infiltrate deep into the layer of underground waters and pollute the groundwater as well as the surface water. Heavy metals in the soil subsequently enter the human food web through plants and they constitute risk to the ecosystem as they tend to bioaccumulate and can be transferred from one food chain to another. Heavy metals are discovered in various food chains where the results are usually detrimental to micro-organisms, plants, animals and humans alike.

Many techniques of remediation of contaminated soil have been developed, such as physical, chemical degradation, photodegradation. However, most of these methods have some drawbacks in completely remediating hydrocarbon contaminated soil. Some of these methods leave behind daughter compounds which are more toxic to the environment than the parent compounds. Biological treatment offers the best environmental friendly method for remediating hydrocarbon and heavy metal contaminated soil because it utilized the capability of the indigenous microorganisms in the soil environment to break down the hydrocarbons and heavy metals into innocuous substances.

Biological remediation, a process defined as the use of microorganisms or plants to detoxify or remove organic and inorganic xenobiotic compounds from the environment is a remediation option that offers green technology solution to the problem of environmental degradation. This process relied upon microbial enzymatic activities to transform or degrade the contaminants from the environment (Philp et al., 2005). It offers a cost effective remediation technique, compared to other remediation methods, because it is a natural process and does not usually produce toxic by-products. It also provides a permanent solution as a result of complete mineralization of the contaminants in the environment (Perelo, 2010). Advantages of biological remediation compared to other treatment methods include (Okoh and Trejo-Hernandez, 2006):

i. Destruction rather than transfer of the contaminants to another medium.
ii. Minimal exposure of workers to the contaminants.
iii. Longtime protection of public health.
iv. Possible reduction in the duration of the remediation process.

2. Impact of hydrocarbon and heavy metals contamination on environment and human health

Hydrocarbon spills in the form of petroleum products both on land and in water, have been a problem since discovery of oil as a fuel source. They can have devastating effects on the biota of an environment. Oil spills and oil waste discharged into the sea from refineries, factories or shipping contain poisonous compounds that constitutes potential danger to plants and animals. The poisons can pass through the food web of an area and may eventually be eaten by humans (Gibson and Parales, 2000).

Environmental contamination by hydrocarbons and petroleum products constitute nuisance to the environment due to their persistent nature and tendency to spread into ground and surface waters. Environmental pollution with petroleum and petrochemical products has attracted much attention in recent decades. The presence of various kinds of automobiles and machinery vehicles has caused an increase in the use of motor oil. Oil spillages into the environment have become one of the major problems. Used motor oils such as diesel or jet fuel contaminate natural environment with hydrocarbon (Husaini, et. al 2008). The hydrocarbons spread horizontally on the groundwater surface thereby causing extensive ground waters contamination (Plohl et al. 2002). Hydrocarbon contamination of the air, soil, freshwater (surface water and groundwater) especially by PAHs has drawn public concerns because many PAHs are toxic, mutagenic, and carcinogenic (Bumpus 1989; Clemente et al. 2001; Cerniglia and Sutherland 2001). Aromatic hydrocarbons are considered to be the most acute toxic component of petroleum products, and are also associated with chronic and carcinogenic effects (Anderson, et al., 1974). Aromatics are often distinguished by the number of rings they possess, which may range from one to five (Anderson, et al., 1974).

Lighter, mono-aromatics (one ring) compounds include benzene, toluene, ethylbenzene, and xylenes (NOAA, 1995). Aromatics with two or more rings are referred to as polycyclic aromatic hydrocarbons (PAHs) (Anderson, et al., 1974). Used lubricating oil contains several toxic components including up to 30% aromatic hydrocarbons, with as much as 22 ppm benzo(a)pyrene (a PAH). Upshall et al (1992) reported that motor oil had a density of 0.828 g/ml and contained 14% aromatics and 65.4% aliphatics (by weight). In their study, the sum of 26 individual PAHs represented 0.17% of the oil, or 1.2% of the aromatic fraction.

The main threats to human health from heavy metals are related with exposure to lead, cadmium, mercury and arsenic (arsenic is a metalloid but is usually classified as a heavy metal). Heavy metals have been utilised by humans for thousands of years. Exposure to heavy metals continues although several adverse health effects of heavy metals have been known for a long time. For example, mercury is still used in gold mining in many parts of Latin America. Arsenic is still common in wood preservatives, and tetraethyl lead remains a common additive to petrol, although this use has decreased dramatically in the developed countries. Waste-derived fuels are especially prone to contain heavy metals which should be a central concern in the consideration for their use. Since the mid 19th century, production of heavy metals increased abruptly for more than 100 years, with associated emissions to the environment, particularly in less developed countries though emissions have lessened in most developed countries over the last century.

Some heavy metals are dangerous to health or to the environment (e.g. mercury, cadmium, lead, chromium), some may cause corrosion (e.g. zinc, lead), some are harmful in other ways (e.g. arsenic may pollute catalysts). Some of these elements are actually necessary for humans in minute amounts (cobalt, copper, chromium, manganese, nickel) while others are carcinogenic or toxic, affecting, among others, the central nervous system (manganese, mercury, lead, arsenic), the kidneys or liver (mercury, lead, cadmium, copper) or skin, bones, or teeth (nickel, cadmium, copper, chromium). One of the largest problems associated with the persistence of heavy metals is the potential for bioaccumulation and biomagnification causing heavier exposure for some organisms than is present in the environment alone. Through precipitation of their compounds or by ion exchange into soils and muds, heavy metal pollutants can localize and lay dormant. Unlike organic pollutants, heavy metals do not decay and thus pose a different kind of challenge for remediation.

3. Remediation techniques for hydrocarbon and heavy metal contaminated soil

Internationally, petroleum contamination is widespread, posing serious environmental risks including surface and groundwater contamination (Balasubramaniam et al. 2007). The environment can potentially be affected by numerous operations in petroleum exploration, production and transportation, with common sources of contamination being leaking underground storage tanks (Nadim et al., 2000). Contamination poses serious environmental risks, including surface and groundwater contamination, and risks to human health and safety (Balasubramaniam et al. 2007). Remediation of contaminated soil is an essential practice. Some of the different techniques used in remediating contaminated soil are discussed below.

3.1 Physical and chemical remediation techniques

3.1.1 In situ soil vapour extraction

Volatile and some semi-volatile organic compounds (VOCs and Semi-VOCs) can be removed from unsaturated soils by a process known as soil vapour extraction (SVE). SVE as an in situ clean-up process allows contaminated soil to be remediated without disturbance or excavation (Nadim et al. 2000).

Soil vapor extraction (SVE) is an in situ unsaturated (vadose) zone soil remediation technology in which a vacuum is applied to the soil to induce the controlled flow of air and remove volatile and some semi-volatile contaminants from the soil. The gas leaving the soil may be treated to recover or destroy the contaminants. The drawback in the use of SVE for remediation of contaminated site is that SVE can not remove heavy oils, metals, PCBs, or dioxins from contaminated soil; it is only effective for remediation of soil contaminated with VOCs and Semi-VOCs. Because the process involves the continuous flow of air through the soil, however, it often promotes the in situ biodegradation of low volatility organic compounds that may be present.

3.1.2 In situ steam injection vapour extraction

Cold soil vapour extraction is a common technique for remediating volatile organic compounds from the unsaturated subsurface. Limitations in efficiency can be overcome by using thermal enhancement, e. g. steam as a fluid heat transport medium to speed up the process (Sleep and Ma, 1997).

In situ steam extraction is a new technology and has had limited use across the globe. Steam extraction can be used in two different systems; mobile and stationary. The mobile system has a unit that volatilizes contaminants in small areas in a sequential manner by injecting steam and hot air through rotating cutter blades that pass through the contaminated medium. The stationary system uses steam injection as a means to volatilize and displace contaminants from the undisturbed subsurface soil. In both systems, steam (at 200^{0}C) and compressed air (at 135^{0}C) are forced through the soil medium and the mixture of air; vapor and chemicals are collected by extraction wells (Nadim et al. 2000).

3.1.3 Air sparging

Air sparging is an in situ technology in which air is injected through a contaminated aquifer. Injected air traverses horizontally and vertically in channels through the soil column, creating an underground stripper that removes contaminants by volatilization (EPA, 2001). Air sparging can also be explained as a method of site remediation that introduces air (or other gases) into the saturated zone contaminated with VOCs. In addition to volatilization of VOCs, air sparging promotes the growth of aerobic bacteria in saturated zones and may oxidize reduced chemical species (Nadim et al 2000). Air sparging has been shown to be effective in removing several types of contaminants such as the lighter petroleum compounds (C_3–C_{10}) and chlorinated solvents (Marley et al. 1992; Reddy et al. 1995).

3.1.4 Excavation

Excavation (removal) is a fundamental remediation method involving the removal of contaminated soil/media, which can be shipped off-site for treatment and/or disposal, or treated on-site when contaminants are amenable to reliable remediation techniques. Excavation is generally utilized for localized contamination and point source and is also used for the removal of underground structures that are out of compliance or have been identified as a potential or actual point source of contamination. The limiting factor for the use of excavation is often represented by the high unit cost for transportation and final off-site disposal. EPA (1991) further stated some limiting factors that may limit the applicability and effectiveness of the process to include:

i. Generation of fugitive emissions may be a problem during operations.
ii. The distance from the contaminated site to the nearest disposal facility will affect cost.
iii. Depth and composition of the media requiring excavation must be considered.
iv. Transportation of the soil through populated areas may affect community acceptability.

In this respect, the on-site removal and treatment can often yield significant savings and, in addition, the treated soil may have beneficial secondary use (e.g. as construction fill or road base material) at the same site.

3.2 Bioremediation techniques

Bioremediation is one of the most viable options for remediating soil contaminated by organic and inorganic compounds considered detrimental to environmental health. Bioremediation is a process defined as the use of microorganisms/plants to detoxify or remove organic and inorganic xenobiotics from the environment. It is a remediation option that offers green technology solution to the problem of hydrocarbon and heavy metals contamination. The main advantage of bioremediation is its reduced cost compared to conventional techniques. Besides cost-effectiveness, it is a permanent solution, which may lead to complete mineralization of the pollutant. Furthermore, it is a non-invasive technique, leaving the ecosystem intact (Perelo, 2010). Bioremediation can deal with lower concentration of contaminants where the cleanup by physical or chemical methods would not be feasible. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. Bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate (Vidali, 2001).

Hydrocarbon considered to be one of the major sources of energy supply across the world usually constitutes major contaminants to both aquatic and terrestrial ecosystems. Various techniques has been employed to remediate soil environment contaminated by hydrocarbons, ranging from physical, to chemical and mechanical forms of treating or removing the contaminants. Bioremediation offers a better technique for treatment and removal of these contaminants into an innocuous substance. Effective bioremediation of hydrocarbons in the soil environment can be achieved by either or both of the following techniques: Biostimulation and Bioaugmentation.

Microorganisms play a significant and vital role in bioremediation of heavy metal contaminated soil and wastewater. Though when microorganisms especially bacteria are exposed to higher concentration of metal, it may have cidal effects on them. Hence, microorganisms are effective only at low metal concentration in the soil. Microorganisms are usually used for the removal of heavy metals. Microorganisms can interact with metals and radionuclides via many mechanisms, some of which may be used as the basis for potential bioremediation strategies (Lloyd et al., 2005). Mechanisms by which microorganisms act on heavy metals includes biosorption (metal sorption to cell surface by physiochemical mechanisms), bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), biomineralization (heavy metal immobilization through the formation of insoluble sulfides or polymeric complexes) intracellular accumulation, and enzyme-catalyzed transformation (redox reactions) (Lloyd, 2002). Biosorption seems to be the most common mechanisms (Haferburg and Knothe, 2007). It is the only option when dead cells are applied as bioremediation agent. However, systems with living cells allow more effective bioremediation processes as they can self-replenish and remove metals via different mechanisms (Malik et al., 2004). On the other hand, living cells shows higher sensitivity to environmental conditions and demand nutritional and energetic sources.

Many genera of microbes like *Bacillus*, *Enterobacter*, *Escherichia*, *Pseudomonas* and also some yeasts and moulds help in bioremediation of metal and chromium-contaminated soil and water by bio-absorption and bioaccumulation of chromium (Kotas and Stasicka, 2000). The heavy metal removal by the bacteria *Pseudomonas* was attributed to the cellular growth of these organisms (Ray and Ray, 2009).

3.2.1 Biostimulation

Biostimulation of indigenous microbes is a bioremediation strategy mostly used for remediation of contaminated soil. This involves addition of nutrients, either organic or inorganic, to enhance the activities of indigenous microbes. Input of large quantities of carbon sources such as crude oil, used lubricating oil, diesel oil etc. tends to result in a rapid depletion of the available pools of major inorganic nutrients such as N and P. Levels of N and P added to stimulate biodegradation at contaminated sites are often estimated from C/N ratios (Sang-Hwan et al. 2007).

Biostimulation aims at enhancing the activities of indigenous microorganisms that are capable of degrading pollutant from soil environment, it is often been applied to the bioremediation of oil-contaminated soil. Nutrient enrichment, also called fertilization is a bioremediation approach in which fertilizer similar to phosphorus and nitrogen that are applied to plant in farms are added to contaminated environment to stimulate the growth of indigenous microorganisms that can degrade pollutants (Thieman and Palladino 2009). Microorganisms need an abundance of key elements such as carbon, hydrogen, nitrogen, oxygen and phosphorus for building macromolecules, addition of fertilizer provides these microbes with essential elements to reproduce and thrive. In some instances, manure, wood chips and straw may provide microbes with the sources of carbon as a fertilizer. The concept of biostimulation is that, by adding more nutrients, microorganisms replicate, increase in number and grow rapidly and thus increase the rate of biodegradation (Thieman and Palladino 2009). Addition of inorganic nutrients do act as fertilizer to stimulate biodegradation by autochthonous microorganisms in some cases; in other cases, it is the intentional stimulation of resident xenobiotic-degrading bacteria by use of electron acceptors, water, nutrient addition, or electron donors (Widada, et al., 2002). Combinations of inorganic nutrients often are more effective than single nutrients (Sutherland, et al., 2000). Laboratory-based respiration experiments by Liebeg and Cutright (1999) showed that a low level of macronutrients and a high level of micronutrients were required to stimulate the activities of indigenous microbes. The greatest stimulation was recorded with a solution consisting of 75% sulphur, 3% nitrogen and 11% phosphorus.

Addition of a carbon source as a nutrient in contaminated soil is known to enhance the rate of pollutant degradation by stimulating the growth of microorganisms responsible for biodegradation of the pollutant. It has been suggested that the addition of carbon in the form of pyruvate stimulates the microbial growth and enhances the rate of PAH degradation (Lee, et al., 2003). Biostimulation can also be achieved by the use of composting bioremediation technologies. Composting bioremediation strategy relies on mixing the primary ingredients of compost with the contaminated soil, such that as the compost matures, the pollutants are degraded by the active microflora within the mixture (Semple, et al., 2001). Mushroom compost and spent mushroom compost (SMC) are also applied in treating organopollutant contaminated sites (Eggen, 1999, Trejo-Hernandez et al., 2001).

Addition of SMC results in enhanced PAH-degrading efficiency (82%) as compared to the removal by sorption on immobilized SMC (46%). It was observed that the addition of SMC to the contaminated medium reduced the toxicity, added enzymes, microorganisms, and nutrients for the microorganisms involved in degradation of PAHs (Lau, et al., 2003). Organic wastes like banana skin, spent mushroom compost and brewery spent grain in earlier studies were found to enhance the biodegradation of used lubricating oil up to 90% loss of oil within the period of 3 months (Abioye, et al., 2009b, 2010). Also the results of our studies revealed the potential of melon shell to stimulate 75% crude oil degradation in soil contaminated with crude oil within the period of 28 days (Abioye, et al., 2009a).

Depending on the nature of the contaminated soil, some of these nutrients could become limiting, hence the additions of nutrients are necessary to enhance the biodegradation of oil pollutants (Choi et al., 2002; Kim et al., 2005). Pelletier et al. (2004) assessed the effectiveness of fertilizers for crude oil bioremediation in sub-Antarctic intertidal sediments over a one-year and observed that chemical, microbial and toxicological parameters demonstrated the effectiveness of various fertilizers in a pristine environment. Frederic et. al., (2005), observed that addition of commercial oleophilic fertilizers containing nitrogen and phosphorus to hydrocarbon contaminated soil increased the hydrocarbon-degrading microbial abundance and total petroleum hydrocarbon degradation, and also reported 77 – 95% loss of total alkanes and 80% of PAHs in hydrocarbons contaminated soil within the period of 180days. In another study using poultry manure as organic fertilizer in contaminated soil, biodegradation was reported to be enhanced in the presence of poultry manure alone, but the extent of biodegradation was influenced by the incorporation of alternate carbon substrates or surfactants (Okolo et al., 2005). However, excessive nutrient concentrations can inhibit the biodegradation activity (Challain et al., 2006), and several authors have reported the negative effect of high NPK levels on the biodegradation of hydrocarbons (Oudot et al., 1998; Chaıneau et al., 2005) and more especially on the aromatics (Carmichael and Pfaender, 1997).

3.2.2 Bioaugmentation

This is an approach that involves introduction of microorganisms that possessed biodegradation potential into the contaminated environment to assist the indigenous microbes with biodegradative processes. This may sometimes involved addition of genetically engineered microorganisms suited for biodegradation of the hydrocarbon contaminants into the contaminated soil. Bioaugmentation is a promising and low-cost bioremediation strategy in which an effective bacterial isolate(s) or microbial consortium capable of degrading xenobiotics is administered to contaminated sites (Gentry et al., 2004). Successful bioremediation of soil contaminated with hydrocarbon sources through bioaugmentation has been reported by various authors. Bagherzadeh et al., (2008) evaluated the efficiency of pollutant removal by selected microorganisms and reported thus: Five mixed cultures and 3 single bacteria strains, *Pseudomonas* sp., *Arthrobacter* sp. and *Mycobacterium* sp. were isolated from hydrocarbon-contaminated soils by enrichment on either crude oil or individual hydrocarbons, as the sole carbon sources. The strains were selected based on their ability to grow in medium containing crude oil, used engine oil or both. Their ability to degrade hydrocarbon contaminants in the environment was investigated using soil samples contaminated with used engine oil. The mixed starter culture #1 degraded 66 % of aliphatic compounds in the engine oil, after 60 days of incubation. The mixed starter culture #5 removed 47 % of aromatic compounds during 60 days of incubation. Bento et al. (2005) reported 72.7% light TPH fraction and 75.2% heavy TPH fraction degradation in diesel contaminated soil bioaugmented with bacterial consortium of *Bacillus cereus, Bacillus sphaericus, Bacillus fusiformis, Bacillus pumilus Acinetobacter junii* and *Pseudomonas* sp. Ying et al. (2010) augmented a PAH-contaminated soil with *Paracoccus* sp. strain HPD-2 and observed 23.2% decrease in soil total PAH concentrations after 28 days, with a decline in average concentration from 9942 to 7638 μg kg^{-1} dry soil. They discovered percentage degradation of 3-, 4- and 5(+6)-ring PAHs was 35.1%, 20.7% and 24.3%, respectively.

The soil environment is very complicated and the degrading ability of exogenously added microorganisms tends to be affected by the physicochemical and biological features of the soil environment. Sometimes, the administration of petroleum degrading microorganisms leads to a failure of bioaugmentation (Vogel 1996; Gentry et al., 2004). Bioaugmentation is not always an effective solution for remediation of contaminated soil because in some cases laboratory strains of microorganisms rarely grow and biodegrade xenobiotics compared to the indigenous microbes (Thieman and Palladino 2009). Also Bioaugmentation is yet to gain public acceptance, most especially the use of genetically engineered microbes due to the believe that these microbes when seeded into contaminated soil may alter the ecology of the environment as well as pose risk to the environmental health if they persist after the remediation of the contaminated soil.

3.3 Phytoremediation of hydrocarbon and metal-contaminated soil

Phytoremediation is a remediation method that utilizes plants to remove, contain or detoxify environmental contaminants (Palmroth, 2006). Phytoremediation appears attractive because in contrast to most other remediation technologies, it is not invasive and, in principle, delivers intact, biologically active soil (Wenzel, 2009). Some major advantages and disadvantages of phytoremediation are shown in Table 1. The most common plant species used in phytoremediation of organic and inorganic compounds includes willows, poplar and different types of grasses. Comprehensive list of plants that has recorded positive results in remediation of organic compounds are listed in Table 2.

On-site phytoremediation of petroleum hydrocarbons and heavy metals can be enhanced by employing a combination of common agronomic practices (e.g. fertilizer application, tillage and irrigation), this is because available nutrient reserves can be quickly depleted as the microbial community begins to degrade the contaminants (Farrell and Germida, 2002). Therefore fertilizer applications may enhance the degradation of petroleum hydrocarbons in soil by reducing competition for limited nutrients. Cutright (1995) reported that increasing the amount of nitrogen and phosphorus in soil under aerobic conditions increased the degradation of PAHs by the soil fungus *Cunninghamella echinulata* var. *elegans.* Brown, (1998) also observed loss of 2- and 3- rings of aromatic hydrocarbons from soil contaminated with weathered petroleum compounds when the soil was amended with sludge compost high in nitrogen compared to no amendment or low nitrogen amendment. Palmroth et al. (2002) recorded 60% loss of diesel fuel in 30 days in diesel-contaminated soil planted with pine tree and amended with NPK fertilizer. Also, Vouillamoz and Milke (2009) observed that compost addition combined with phytoremediation, increases the rate of removal of diesel fuel in soil. Agamuthu et al., (2010) recorded appreciable degradation of used lubricating oil in soil when the growth of *Jatropha curcas* was enhanced with brewery spent grain.

Advantages	Disadvantages
Relatively low cost	Longer remediation time
Easily implemented and maintained	Climate dependent
Several mechanisms for removal	Effects to food web might be unknown
Environmentally friendly	Ultimate contaminant fate might be unknown
Aesthetically pleasing	Results are variable
Reduces landfilled wastes	
Harvestable plant materials	
Costs 10 – 20% of mechanical treatments	Slower than mechanical treatments
Faster than natural attenuation	Only effective for moderately hydrophobic compounds
High public acceptance	Toxicity and bioavailability of biodegradation products is not known.
Fewer air and water emissions	Contaminants may be mobilized into the ground water
Conserves natural resources	Influenced by soil and climate conditions of the site.
(Susarla et al., 2002; Kamath, et al., 2004)	

Table 1. Advantages and disadvantages of phytoremediation over traditional technologies.

3.3.1 Mechanisms of phytoremediation

Variety of pollutant attenuation mechanisms possessed by plants makes their use in remediating contaminated land and water more feasible than physical and chemical remediation (Glick, 2003; Huang et al., 2004, 2005; Greenberg, 2006; Gerhardt et al., 2009). As a result of their sedentary nature, plants have evolved diverse abilities for dealing with toxic compounds in their environment. Plants act as solar-driven pumping and filtering systems as they take up contaminants (mainly water soluble) through their roots and transport/translocate them through various plant tissues where they can be metabolized, sequestered, or volatilized (Greenberg et al., 2006; Abhilash, 2009). Plants utilize different types of mechanisms for dealing with environmental pollutants in soil. The mechanisms of phytoremediation include biophysical and biochemical processes like adsorption, transport and translocation, as well as transformation and mineralization by plant enzymes (Meagher, 2000). Plants have been shown to be able to degrade halogenated compounds like TCE by oxidative degradation pathways, including plant specific dehalogenases (Nzengung, et al., 1999). Dehalogenase activity was observed to be maintained after the plants death. Enzymes can become bound to the organic matrix of the sediment as plants die, they decay and they are buried in the sediment, thus contributing to the dehalogenase activity observed in organic-rich sediments (Nzengung, et al., 1999).

Plant used	Contaminants	Results	Reference
Jatropha curcas	Coal fly ash, lead, cadmium, arsenic and chromium	Enhanced heavy metals uptake by 117% in root, 62% in stem and 86% in leaves when EDTA was applied at 0.3g/kg to fly ash. Jatropha accumulated Cd and Pb in the shoot. It shows increase bioaccumulation potential of As and Cr with increase in metal concentration in soil system.	Santosh et al., (2009) Jamil et al., (2009) Mangkoediharjo and Surahmaida (2008)
Carex exigua, Panicum virgatum Tripsacum dactyloides	Petroleum hydrocarbons	70% loss of total petroleum hydrocarbons was recorded after one year growth of these plants in contaminated soil.	Euliss et al., (2008)
Vicia faba	Crude petroleum oil	47% of total petroleum hydrocarbon was degraded in 60 days.	Diab (2008)
Populus tremula	Cadmium and Zinc	Both Cd and Zn accumulated in the leaves with maximum foliar concentration of 35 and 2400mg/kg	Hassinen et al., (2009)
Ditch reed and Alfalfa	Liquid bitumen agar (mainly paraffins & naphtenes) 70.9g/kg and soil containing PAHs 80mg/kg	82% removal was achieved in 27 months with both plants.	Muratova et al., (2003)
Tall fescue	PAHs in creosote contaminated soil.	Removal of acenaphtene and fluorine in 36 months was slightly higher in the presence of tall fescue than in unvegetated soil.	Robinson et al., (2003)
Rye grass and Sweet clover	Aged PAHs from manufacture gas plant.	PAHs removal in 12 months was higher in the presence of plants, 9% to 24% compared to 5% without plant.	Parish et al., (2004)

Table 2. Examples of plants used for phytoremediation of organic contaminants.

Variety of contaminant-degrading enzymes can be found in plants. These include peroxidases, dioxygenases, P450 monooxygenases, laccases, phosphatases, dehalogenases, nitrilases, and nitroreductases (Susarla et al., 2002; Singer et al., 2004 Chaudhry et al., 2005). Phytoremediation is based upon the basic physiological mechanisms taking place in higher plants and associated microorganisms, such as transpiration, photosynthesis, metabolism, and mineral nutrition. Plants dig their roots in soils, sediments and water, and roots can take up organic compounds and inorganic substances; roots can stabilize and bind substances on their external surfaces, and when they interact with microorganisms in the rhizosphere (Marmiroli et al., 2006). Uptaken substances may be transported, stored, converted, and accumulated in the different cells and tissues of the plant. Finally, aerial parts of the plant may exchange gases with the atmosphere allowing uptake or release of molecules (Marmiroli et al., 2006). A series of six phytotechnologies have been identified by Interstate Technology and Regulatory Cooperation (ITRC, 2001) which may address different contaminants in different substrates, and which rely on one or more of the plant properties.

1. Phytotransformation, ideal for organic contaminants in all substrates
2. Rhizosphere bioremediation, applied to organic contaminants in soil
3. Phytostabilisation, for organic and inorganic contaminants in soil
4. Phytoextraction, useful for inorganic contaminants in all substrates
5. Phytovolatilisation, which concerns volatile substances
6. Evapotranspiration, to control hydraulic flow in the contaminated environment

4. Conclusion

Remediation of hydrocarbon contaminated soil is a necessity in order to have a safe and healthy environment that will in turn results in healthy lifestyle across the globe. Biological remediation of hydrocarbon and metal contaminated soil offers a better and more environmentally friendly technique that if properly and thoroughly explored can bring our environment into a better place for both plant and animals well being due to its enormous advantages over other treatment methods. However, despite these enormous advantages of biological treatment method, its potential is yet to be fully utilized in restoration of contaminated soil. This is possibly due to the fact that it takes a longer period of time for the complete restoration of the environment; this limitation can however be overcome through nutrient addition and introduction of microbes with biodegradative capability to degrade hydrocarbon and heavy metals in the environment. Future research and developments will requires focus on the use of cheap, environmental friendly and widely available nutrients that can be used to enhance the microbial and plant activities in mineralizing hydrocarbons and heavy metals in soil environment.

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8

Bioindicators and Biomarkers in the Assessment of Soil Toxicity

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1. Introduction

Several potentially harmful chemical compounds, derived from activities of urban centres, rural properties and industries are constantly released into the terrestrial environment. In this context, the scientific community has shown increasing interest in the detection, knowledge and control of environmental agents responsible for damages to human health and sustainability of ecosystems (Magalhães & Ferrão-Filho, 2008).

Monitoring the types and quantities of toxic substances that are entering into the terrestrial environment is an exhaustive and problematic task due, mainly, to the complexity and cost resulting from the identification of the chemical substances involved. Despite the numerous analytical methods available, collecting sufficient samples in a timely fashion continues to be a great obstacle in the evaluation of environmental damages (Silva et al., 2003).

Furthermore, the determination of isolated substances by traditional chemical analyses has a limited environmental application, since it does not detect the effects on the organisms neither inform about the possible interactions between the substances (additive, antagonistic or synergistic), as well as their bioavailability (Magalhães & Ferrão-Filho, 2008). In this sense, researchers have pointed the necessity to apply biological methodologies in order to obtain an ecosystemic approach.

Biological factors may indicate better the environmental balance through the biotic indexes, derived from the observation of bioindicator species. According to Hodkinson and Jackson (2005), it is called bioindicator a species or group of species that reflects biotic and abiotic levels of contamination of an environment, presenting alterations that enables the generation of information about the quality of the environment, for example, accumulating substances in concentrations higher than those considered normal or essential for its body metabolism or presenting alterations in the number of organisms. Such organisms, due to their characteristics of little ecological tolerance to some chemical substances, can present some alteration, whether it is physiological, morphological of behavioural, when exposed to certain pollutants (Magalhães & Ferrão-Filho, 2008).

Due to their close contact with soil, some taxonomic groups of invertebrates belonging to the meso- and macro-fauna such as, Isopoda, Collembola, Oligochaeta and Diplopoda, have been proposed as bioindicator organisms (Hopkin, 2002). In addition, higher plants such as *Allium cepa* (onion), *Arabidopsis thaliana* (mustard), *Hordeum vulgare* (barley), *Tradescantia sp.*, *Vicia faba* (broad bean) and *Zea mays* (corn) are also commonly used in the assessment of soil toxicity, particularly for cytotoxicity, genotoxicity and mutagenicity assays (White & Claxton, 2004).

In a biological system, the sequential order of alterations promoted by the presence of pollutants occurs in crescent levels of biological organization, extending from the molecular or biochemical level to the physiological or individual level, until the population and ecosystem level (Stegeman et al., 1992). When a significative alteration is evident, the ecosystem must be already severely damaged. Therefore, techniques that show responses at lower levels of biological organization are considered more preventive (Nascimento et al., 2008).

Morphological alterations can be used as biomarkers in toxicity investigations of specific chemical compounds and in the monitoring of the acute and chronic effects of organisms exposed to impacted environments. In this context, the morphological analysis of target organs, carried out by ultra-morphology, histology and ultrastructure, has become widely used in studies with invertebrates, aiming to identify different damages caused by harmful substances to the organisms (Fontanetti et al., 2010).

Another tool that has shown to be increasingly efficient in the assessment of soil toxicants on the organisms is the use of molecular biomarkers. Recent studies show great interest in the use of enzymatic biomarkers as a form to monitor the environment, since the increase or inhibition of the activity of certain enzymes can explain a possible response to the environment stress.

Due to the importance to ensure the genetic integrity of the organisms, biomarkers of genotoxicity are gaining attention in the evaluation of the toxic potential of soil samples (Misik et al., 2011). The tests used in the genotoxic assessment of an agent (genotoxicity and mutagenicity tests), include the Ames test, chromosome aberrations test, micronucleus test, comet assay, SMART test (Somatic Mutation and Recombination Test), microarray and microscreen, using techniques of cellular, molecular and genetic biology both *in vitro* and *in vivo*, *in situ* and *ex situ*.

Given the above, the aim of the present chapter was to compile and discuss information present in the literature about the use of animal and plant bioindicators in the analysis of soil toxicity, as well as characterize the different biomarkers used in these organisms that enable the assessment of the soil toxicant effects in different levels of the biological scale, i.e., morphological, biochemical and genotoxic.

2. Complex substances, organic compounds and metals: potential soil contaminants

Population growth combined with the increasing industrialization is responsible for generating tons of waste per day, which, many times, are accumulated in the environment without any previous treatment. Soil becomes a cheaper and practice alternative for the final disposal of these residues, but not without consequences. Soil contamination is a broad problem, since the contaminants can be leached into groundwater, rivers and lakes. The major xenobiotics responsible for the contamination of this compartment as well as their implications for invertebrates and plants will be discussed.

2.1 Vinasse

Among the substances released into the soil with toxic potential it can be cited the vinasse, a product of the alcohol production, composed by water (97%) and a solid fraction (organic matter and mineral elements). According to Sahai et al. (1985), due to the fast growth of the distilleries, there was, consequently, an increase in the amount of this residue, which was traditionally discharged in open areas or next to water courses, causing air, water and soil pollution. For Junior et al. (2008), the reuse of previously treated vinasse as fertilizer or soil conditioner becomes an alternative of great interest. However, Lyra et al. (2003) affirm that there are only few studies that evaluate its pollutant potential on soil and groundwater.

Thus, studies about the effects of vinasse application in the soil have been developed by researchers of different countries. Recently, Brazilian researchers have studied the genotoxicity and mutagenicity of vinasse applied *in natura* in the soil or associated with other compounds. Souza et al. (2009) used *A. cepa* to evaluate the mitotic and chromosome abnormalities resulted from exposure to landfarming soil treated with vinasse, used as a possible bioremediator. According to the authors, the vinasse was responsible for potentiating the clastogenicity of the landfarming by decreasing the pH and, thus, making available the metals that were strongly adsorbed in the organic matter of the soil.

Other studies on the vinasse toxicity were conducted by Christofoletti and Fontanetti (2010) and Pedro-Escher and Fontanetti (2010) also using *A. cepa* as test organism. In the first study, the preliminary results show that the vinasse did not present cytotoxicity nor mutagenicity, but it presented genotoxic potential when applied *in natura* or associated with sewage sludge samples; the second study showed that different concentrations of vinasse diluted in water (12.5%, 25% and 50%) presented genotoxic potential and only the raw vinasse presented mutagenic potential, thus suggesting that the vinasse can cause damages in the genetic material of certain organisms.

2.2 Sewage sludge

Another residue with pollutant potencial and problems in final disposal is the sewage sludge generated in the STSs (Sewage Treatment Stations). Its great production, mainly in the large urban centres, has led researchers to intensify the studies about the use of these waste with agricultural purposes. Therefore, recycling, via agriculture use, presents itself as a global trend (Lopes et al., 2005).

Nevertheless, sewage sludge can present, in its composition, undesirable chemicals (metals and organic chemical compounds) and biological elements (pathogens) that, in contact with man and/or fauna and flora, may cause contamination and diseases. Thus, any decision on the most appropriate final destination depends on the evaluation and minimization of the contamination risks of the environment and man (Rocha & Shirota, 1999).

Studies involving millipeds exposed to sewage sludge have shown that its components can affect the integrity of organs such as the midgut of these animals (Godoy & Fontanetti, 2010; Nogarol & Fontanetti, 2010, 2011; Perez & Fontanetti, 2011a). Mazzeo et al. (2010), using *A. cepa,* investigated the genotoxic and mutagenic potential of domestic sewage sludge at different concentrations.

2.3 Polycyclic aromatic hydrocarbons (PHAs)

Some of the main pollutants that cause concern in relation to soil contamination are the PAHs (Bispo et al., 1999), which are compounds formed by two or more benzene rings, exclusively constituted by atoms of carbon and hydrogen (Netto et al., 2000). There are many origin sources but it can be highlighted industrial processes, such as petroleum refining, combustion of organic matter and burning of coal (Page et al., 1999). According to the IUPAC (International Union of Pure and Applied Chemistry) there are, currently, over

100 known PAHs, however only 16 have environmental and toxicological importance (Environment Protection Agency [EPA], 1986). These compounds are able to react, directly or after undergoing metabolic transformations, with the DNA, becoming potential carcinogens and efficient mutagens.

Soil receives considerable amounts of PAHs that, because of the complexity of their chemical structure, low solubility in water and strong sorption tendency into the soil, become recalcitrant and remain for long periods in the environment, enhancing the probability of exposure of humans and animals to these compounds (Jacques et al., 2007). When present in the environment they can be transferred to invertebrates by ingestion of soil and plant material contaminated or by cuticle (Achazi & Van Gestel, 2003).

In order to reduce the negative impact of these compounds in the soil, petroleum refineries use a bioremediation system called landfarming. The technique has been used for the treatment of soils contaminated with hydrocarbons for 100 years and, the petroleum industry for at least 25 years (Riser-Roberts, 1998). Currently, other types of industry started to employ this technique, such as textile and food industries and treatment of effluents.

Souza et al. (2009) carried out bioassays with *A. cepa* in order to assess landfarming soil samples before and after biodegradation of hydrocarbons. Before biodegradation, the landfarming had 13.5 g/Kg of Total Petroleum Hydrocarbons (TPH) and caused strong clastogenic and mutagenic effects. After 108 days of biodegradation, the concentration of TPH decreased 27% with significant reduce of mitotic and chromosome abnormalities, micronuclei and nuclear buds.

Using the diplopod *R. padbergi* exposed to different concentrations of industrial soil contaminated with PAHs, Souza et al. (2011) and Souza and Fontanetti (2011) analyzed the perivisceral fat body and midtgut of the animals and verified that there were several alterations in these two tissues.

2.4 Dioxins

Seven dibenzo-p-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs) and 12 polychlorinated biphenyls (PCBs) are called dioxins (Word Health Organizatrion [WHO], 2010; United States Environmental Protection Agency [USEPA], 2000, 2003), being released into the environment as a byproduct of chemical processes and through the combustion of industrial and municipal wastes (Stephens et al., 1995). According to Schlatter (1994), since the accident in Seveso, Italy, they became the symbol of threat caused by toxic chemicals. As a result of widespread fear, dioxins are a matter of real concern in relation to environmental contamination.

Among the isomers of PCDDs, the most toxic is 2,3,7,8 - tetrachloro-dibenzo-para-dioxin (2,3,7,8-TCDD) (Eisler, 1986). There is little information in literature on the effects of PCDDs on terrestrial invertebrates. Reinecke and Nash (1984) reported that two species of earthworms (*Allolobophophora caliginosa* and *Lumbricus rubellus*) showed no adverse effects when exposed for 85 days in soil with 5 ppm of 2,3,7,8-TCDD, but both species died at 10 ppm. Studies involving plants and PCBs have been made, since these organisms are less sensitive to PCBs and thus may be a possible route of biomagnification in various food chains (Sinkkonen et al., 1995).

2.5 Agrochemicals

The use of fertilizers and pesticides has become a common practice due to population growth, food crisis and consequent need for the increase in the agriculture production.

Within the existing agriculture model, agrochemicals are classified as one of the main chemical pollutants that are disseminated throughout the planet (Grisolia, 2005). However, there is still little information about the effects of these chemical compounds on invertebrates that occupy levels of high sensitivity in trophic chain (Mantecca et al., 2006).

Among the studies carried out with terrestrial invertebrates it was analyzed the possible alterations in the biomass (Niemeyer et al., 2006a), reproduction (Helling et al., 2000), behaviour (Niemeyer et al., 2006b), survival (Diao et al., 2007) and tissular and cellular lesions (Nasiruddin & Mordue, 1993) resulted from exposure to certain agrochemicals. Associated with the use of bioindicators and biomarkers it is also used the direct analysis of the presence of residues in soil samples by specific equipments such as the spectrophotometer or chromatograph.

Pesticides have been widely tested by bioassays with plants and positive results are usually obtained (Leme & Marin-Morales, 2009). A clear example is the *A. cepa* test successfully used in the evaluation of the mutagenic and genotoxic potential of herbicides such as trifluralin (Fernandes et al., 2007; 2009).

2.6 Metals

Heavy metals or trace metals are terms applied for a great amount of trace elements that are industrially and biologically important. From the point of view of human health, agriculture and ecotoxicology, the most worrying heavy metals are As, Cd, Hg, Pb, Ti and U. Studies involving heavy metals in ecosystems have shown that many areas near urban centres, mines and road systems have high concentrations of these elements (Alloway, 1994). Metals are highly persistent in the soil with persistence of up to thousands of years (McGrath, 1987) and can express their pollutant potential directly on the soil organisms by availability to plants and transference to the food chain, both by plants and by the contamination of superficial waters or groundwater (Chang et al., 1987).

The main anthropogenic sources of metals are fertilizers, pesticides, contaminated irrigation water, combustion of coal and oil, vehicular emissions, incineration of urban and industrial wastes and, mainly, mining and smelting (Tavares & Carvalho, 1992).

Due to their habits in the superficial layers of the soil, invertebrates of the saprophagous fauna, such as isopods, diplopods and springtails are regularly exposed to metals (Hopkin, 2002). Heikens et al. (2001) carried out a literature review to clarify the concentration of metals in terrestrial invertebrates and they concluded that the concentration in most of the groups happened in the order Pb>Cd>Cu. Afterwards, Köhler (2002) conducted a study to determine the location of these metals in the bodies of soil arthropods. The genotoxic potential of metals has also been studied by several authors using plants as test systems (Knasmüller et al., 1998; Rank & Nielsen, 1998).

3. Invertebrates of the edaphic fauna and higher plants as soil bioindicators

One important question in ecotoxicological studies refers to the choice of the bioindicator species. It will depend on its ecological and toxicological importance, facility to be maintained in laboratory, reproductive rate and sensitivity (it must be affected by several chemical agents but less affected by abiotic factors) (Römbke & Garcia, 2000). Many authors agree that the main features needed to be a good bioindicator are sensitivity, good representativeness and functional importance in the ecosystem, as well as easy collection, identification and analysis (Greensdale, 2007). In this context, some taxonomic groups of soil invertebrates and higher plants have been proposed as bioindicator organisms.

3.1 Terrestrial invertebrates

Terrestrial arthropods of the saprophagous fauna such as, Isopoda, Collembola and Diplopoda are among the most appropriate organisms to evaluate the effects of the accumulation of toxic substances present in the soil, due to their direct contact with contaminants present in it (Gräff et al., 1997; Hopkin et al., 1989). Annelids, in special the Oligochaeta, are also frequently used in toxicity tests. These invertebrates get in contact with a great variety of pollutants present in this compartment by their movement and ingestion of contaminated soil or leaf litter (Spadotto et al. 2004).

Oligochaeta are considered one of most important representatives of the edaphic macrofauna (Kale, 1988). Several factors make earthworms excellent bioindicators of the toxicity of chemical substances in the soil, such as the knowledge already accumulated on their habitats and important trophic position of these invertebrates, which are situated in the lowest levels of the terrestrial food webs, serving as food for several animals and route of transference and biomagnification of contaminants along these webs (Andréa, 2010).

Due to their great importance in the soil, their wide distribution and all the reasons previously cited, earthworms, mainly the species *Eisenia fetida* (figure 1) and *E. andrei* were chosen for several toxicity tests for registration of agrochemicals in the regulatory agencies of several countries, including Brazil (Andréa, 2010). Other species such as *Lumbricus terrestris* and *L. rubellus* have been widely used in studies of bioaccumulation of metals (Amaral & Rodrigues, 2005; Veltman et al., 2007).

Fig. 1. Earthworm *Eisenia fetida.* (Photo: Raphael Bastão de Souza and Larissa Rosa Nogarol)

Collembola are among the most important members of the soil meso-fauna involved in the decomposition process and are vulnerable to the effects of its contamination (Bengtsson & Rundgren, 1984). Greensdale (2007) lists some favourable points in choosing Collembola as bioindicators, such as presence in all ecosystems, abundance and ease of collection in sufficient number to allow statistical analyses. Moreover, they have short life cycle, making that they respond quickly to environmental changes and, as they are in direct contact with the soil, they are more sensitive to some type of stress applied in the ecosystem.

Several studies point out this organism as bioindicator, applying different methodologies and evaluation parameter. Tests of reproduction associated to survival rates (Pedersen et al., 2009; Sverdrup et al., 2010;) and evaluation of abundance and/or diversity of species in areas that suffer some type of degradation (Sousa et al., 2004) are the most used methodologies.

Another taxonomic group used in toxicological analyses is Isopoda, one of the largest orders of crustaceans with approximately 10,000 thousand described species, mostly marine

(Schultz, 1982). Terrestrial isopods have already been used in toxicity tests of soil and the main parameters of evaluation were abundance of individuals (Faulkner & Lochmiller, 2000), reproduction rates (Niemeyer et al., 2009) and survival (Stanek et al., 2006).

Metals are the main toxic agents evaluated using Isopoda, since these invertebrates bioaccumulate these elements. In this sense, researchers have carried out studies on bioaccumulation (Blanusa et al., 2002; Hopkin et al., 1993), the cytotoxic effect of metals (Köhler et al., 1996a; Odendaal & Reinecke, 2003) and the detoxification mechanisms (Hopkin, 1990; Köhler & Triebskorn, 1998) and the terrestrial isopod *Porcellio scaber* is the most studied.

The importance of diplopods in the recycling of nutrients, aeration and fertilization of soil is frequently mentioned in the literature (Dangerfield & Telford, 1989). Due to the habits of the diplopods, colonizers of various soil layers, these animals can be greatly influenced by the deposition of metals, organic compounds and complex substances in the soil.

Most studies in the literature using diplopods as bioindicators of the soil are related to metals. However, the effect of organic pollutants and complex mixtures on these invertebrates is relatively little known (Souza & Fontanetti, 2011). In this context, the first study carried out with diplopods, as possible bioindicators, was conducted by Hopkin et al. (1985), involving the assimilation of metals by the species *Glomeris marginata*. In this study, it was verified a higher uptake of copper, zinc and cadmium by the animals collected in soils contaminated when compared to those animals collected in non-contaminated environments. The authors comment that ultrastructural studies of different organs would be necessary to understand the “metals path”, particularly in the gut of these invertebrates.

Later, Triebskorn et al. (1991) exposed several invertebrates such as mites, insects and diplopods to different toxic substances and used the ultrastructural analysis in order to demonstrate the applicability of using such animals in biomonitoring. In the study carried out by Köhler et al. (1992), it was analyzed the impact of lead on the efficiency of assimilation in diplpods, submitted to different environmental conditions. The researchers used different species of diplopods and found that only *Glomeris conspersa* increased the ingestion of food containing lead when compared to a non-contaminated diet.

Recently, the toxicity assessment of complex substances was performed with the Brazilian species *Rhinocricus padbergi* (figure 2) exposed to different concentrations of sewage sludge (Godoy & Fontanetti, 2010; Nogarol & Fontanetti, 2010, 2011; Perez & Fontanetti, 2011a) and landfarming (Souza & Fontanetti, 2011; Souza et al., 2011). The histological and histochemical analysis, as well as the ultrastructural analysis, showed that such substances are toxic to the diplopod studied, since different tissular and cellular alterations were observed in the midgut and perivisceral fat body of these invertebrates.

Fig. 2. Diplopod *Rhinocricus padbergi*. (Photo: Larissa Rosa Nogarol and Raphael Bastão de Souza)

3.2 Higher plants

Plants, despite their structure and metabolic differences, can offer important information about the cytotoxic, genotoxic and mutagenic potential of substances, even when exposed in short term and offer some advantages such as low cost cultivation and easy maintenance, comparatively, to mammals (Rodrigues et al., 1997). In studies with complex mixtures, plants have also shown satisfactory results, indicating that plants are sensitive enough to detect the adverse effects of environmental samples (Majer et al., 2005).

Plants can be directly exposed to the contaminant, without any dilution or filtration of the sample (Steinkellner et al., 1999). Moreover, Grant (1994) cites other advantages of employing higher plants: (1) higher plants are eukaryotes, thus, their structure and cellular organization are similar to that of humans and it is possible to establish comparisons with animals; (2) the techniques employed for the study are relatively simple and can be performed with agility; (3) cultivation of the organisms has low cost and easy maintenance; (4) the assays can be carried out under a wide range of environmental conditions, pH and temperature; (5) higher plants can regenerate easily; (6) assays with higher plants can be used to assess the genotoxic potential of simple substances or even complex mixtures; (7) it can be used for *in situ* monitoring; (8) can be used for monitoring for several years and are highly reliable; (9) studies have shown correlations with cytogenetic assays in mammals; (10) can be used together with microbial assays to detect mutagenic metabolites (pro-mutagens); (11) genotoxicity studies with plants are presenting high sensitivity in tests with carcinogenic agents.

On the other hand, according to Majer et al. (2005), one of the limitations of using plants as bioindicators is the lack of sensitivity for certain classes of pro-mutagens such as the nitrosamines, heterocyclic amines and some classes of PAHs. In contrast, Ventura (2009) showed that the *A. cepa* system is susceptible to nitro aminobenzene, while Mazzeo (2009) observed the same effect for benzene, toluene, ethylbenzene and xilene (BTEX).

Among the higher plants, onion (*A. cepa*) is the most used plant to determine the cytotoxic, genotoxic and mutagenic effects of many substances present in the soil. Its cellular kinetics characteristic favours a rapid growth of the roots, due to the great number of cells in division. Therefore, the record of the mitotic activity and abnormalities in the cell cycle of the meristematic cells of its roots can be easily visualized (Grant, 1994). Leme and Marin-Morales (2009) affirm that the *A. cepa* test is a fast and sensitive technique to detect genoxotic and mutagenic substances dispersed in the environment.

The evaluation of genetic alteration can be also performed using different species of the genus *Tradescantia* (figure 3) through the detection of mutations induced by agents present in the air, soil and water by the analysis of micronuclei in the mother cell of the pollen grain (Trad-MCN). The species *Tradescantia* are specially indicated for direct application in regions and countries in development due to the advantages such as easy handling and relatively low maintenance cost (Shima et al., 1997).

Vicia faba is a popular material that has been widely used not only in cytological studies, but also in physiological experiments (Kanaya et al., 1994). This organism was initially used in radiobiological tests in investigation of mechanisms of formation of chromosomal aberrations by ionizing radiation (Read, 1959). Later, Kihlman (1975) developed and standardized the *V. faba* meristematic cell bioassay for analysis of chromosomal aberrations, and since then has been widely used for genotoxicity studies for evaluation of sister chromatid exchange (Kihlman & Kronborg, 1976; Kihlman & Andersson, 1984). This technique is very similar to *A. cepa* test; the method does not require sterile conditions or any material or equipment of high cost. Further details of this test are described by Kihlman (1975).

Fig. 3. *Tradescantia pallida*. (Photo: Guilherme Thiago Maziviero)

4. Biomarkers

Molecular, biochemical and physiological compensatory mechanisms can become operative in organisms after exposure to environmental contaminants. This may result in the inhibition or facilitation of one or more physiological mechanisms or functional and structural changes. In this sense, the use of biomarkers allows obtaining information about the biological effects of pollutants and mechanisms of action of xenobiotics on the fauna.

Several authors have proposed different definition for the term biomarkers. According to Lam and Gray (2003), biomarkers can be defined as biochemical, cellular or molecular alterations or physiological changes in the cells, body fluids, tissues or organs of an organism that are indicative of exposure or effect of a xenobiotic. Despite being older, the definition proposed by Depledge (1993) and Depledge et al. (1993) has a more comprehensive character and it is considered the most widely used nowadays: biomarkers are defined as adaptive biological responses to stressors, evidenced as biochemical, cellular, histological, physiological or behavioural alterations.

In the scope of measuring the toxic effects in the organisms at a cellular or molecular level, biomarkers represent an initial response to environmental disturbances and contamination. Therefore, they are generally considered more sensitive than the tests that measure these effects at higher levels of biological hierarchy, such as individual or population (McCarthy & Shugart, 1990).

Thus, during the last decades, several biomarkers have been used effectively, especially as tests for specific toxicants, since biomarkers when combined with biomonitors can create a sophisticated multiple target system to detect a variety of environmental hazards in a fast and economically feasible way, in a single test organism, helping in the establishment of priorities for action in the control of environmental pollution.

4.1 Morphological biomarkers

The detection of many classes of damage in several tissues and cellular types becomes possible by using morphological biomarkers. Such morphological alterations may provide qualitative evidences of a functional adaptation to the external environment (Meyers & Hendricks, 1985). Moreover, the qualitative assessment of such changes before the death of the organism may provide early indications of toxicity (Nogarol & Fontanetti, 2010; Triebskorn et al., 1999).

For such analysis, histology and ultrastructure are used. By these techniques it is possible to diagnose cellular and sub-cellular symptoms resulted from intoxication as well as locate symptoms of cellular death and reveal reactions in response to chronic and sub-lethal exposure in cells and tissues (Fontanetti et al., 2010; Kammenga et al., 2000).

Studies show that one of the main contaminants of the soil, metals, are selectively concentrated in only one or few organs, or in specific regions of the tissues in most of soil invertebrates and typically these organs are part of the digestive tract (Dallinger, 1993). For example, in millipeds (Köhler & Alberti, 1992), isopods (Dallinger & Prosi 1988) and springtails (Pawert et al., 1996), the epithelium of the midgut is the main target of metals. Thus, the epithelium of the digestive tract represents the first barrier against the intoxication of the whole organism (Walker, 1976).

In diplopods, some studies with this approach were performed using the digestive tube and the fat body (Hopkin et al., 1985; Köhler & Triebskorn, 1998; Triebskorn et al., 1999). Morphological alterations observed in the midgut (figure 4) and in the perivisceral fat body (figure 5) of the diplopod *R. padbergi* were successfully used as sublethal biomarkers in the evaluation of soils contaminated with complex substances such as sewage sludge (Godoy & Fontanetti, 2010; Nogarol & Fontanetti, 2010, 2011; Perez & Fontanetti, 2011a) and landfarming (Souza & Fontanetti, 2011; Souza et al., 2011).

In the studies performed with the diplopod *R. padbergi*, it was possible to observe tissular and cellular responses related to detoxification mechanisms such as increased cytoplasmic granules (spherocrystals) and intense release of secretory vesicles into the intestinal lumen of these invertebrates (Nogarol & Fontanetti, 2010; Perez & Fontanetti, 2011a). These secretory vesicles of the apocrine type seems to help in the detoxification of toxic substances initially absorbed by the organism and form a protector layer that would reduce the contact between the toxic agent and the intestinal epithelium.

The formation of agglomerates of haemocytes through the cells of the "fat body" layer was also observed and this response is directly related to a defence mechanism of the animal. According to van de Braak (2002), haemocytes can migrate to the injury site in the tissue by a chemotaxis process that results in inflammation. By this inflammatory reaction, these cells act in the removal of toxins and possibly help in the re-absorption of the damaged epithelium in order to maintain the homeostasis of the organism. In a recent review conducted by Perez and Fontanetti (2011b) it becomes clear that this tissular response is common in different invertebrates exposed to environmental stress conditions. According to the authors, the monitoring of the number of haemocytes can be used as a measure of stress in sentinel species due to environmental contamination.

The mechanisms of defence and detoxification require high and continuous energy expenditure, especially when the organism is exposed to a toxic agent for a long period. In this sense, histological and ultrastructural studies showed some of the main responses of this invertebrate related to higher energetic needs. Nogarol and Fontanetti (2011) observed at an ultrastructural level a high increase in the number of tracheioles between the cells of the "fat body" layer that compose the midgut of diplopods sub-chronically exposed to sewage sludge. The authors suggest that a higher oxygenation of the tissue was necessary to enable the formation of molecules of adenosine triphosphate (ATP), used in the detoxification mechanisms.

Toxic agents may be able to cause cellular death by necrosis, evidenced mainly by the intense cytoplasmic vacuolization in the principal cells of the midgut epithelium of diplopods exposed to landfarming (Souza & Fontanetti, 2011) and sewage sludge (Nogarol & Fontanetti, 2011; Perez & Fontanetti, 2011a). In addition to the cytoplasm, other cellular compartments were affected by the exposure to toxic agents leading to cellular inviability. In these cases, damaged cells are expelled towards intestinal lumen.

Samples of landfarming and sewage sludge presented genotoxic action, evidenced by the occurrence of nucleus fragmentation in the principal epithelial cells, karyolysis in the nucleus of the cells of the fat body layer (Souza & Fontanetti, 2011) and loss of integrity of the nuclear envelope of hepatic cells and cells of the "fat body" layer of the midgut (Nogarol & Fontanetti, 2011).

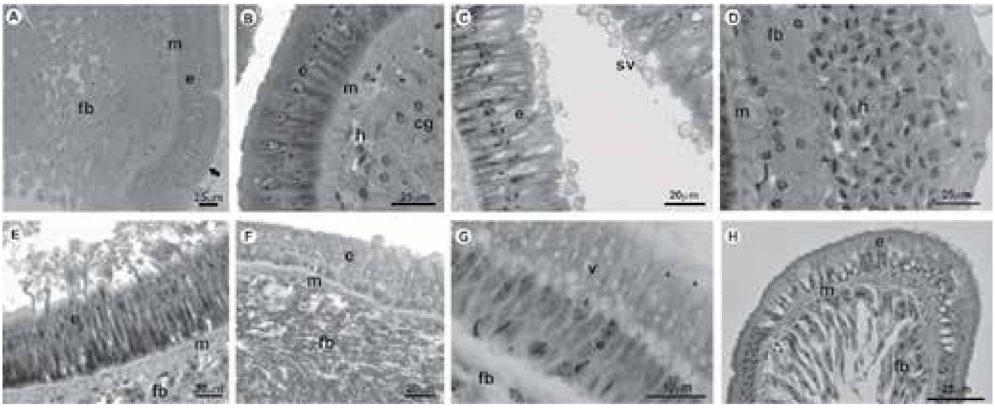

Fig. 4. Midgut of the diplopod *R. padbergi* stained with Hematoxylin-Eosin. Unexposed animals (A; B); Animals exposed to sewage sludge (C-H). secretion vesicles (C); haemocytes aglomeration (D); epithelium renewal (E); increase of cytoplasmatic granules in "fat body layer" (F); cytoplasmatic vacuolization (G); volume reduction of the cells in "fat body" layer of midgut (H). e=epithelium; m= muscle layer; fb= "fat body" layer; h= haemocytes; v= vacuole; sv= secretion vesicle; * dilatation of intercellular space (Photos: Larissa Rosa Nogarol; Raphael Bastão de Souza and Tatiana da Silva Souza)

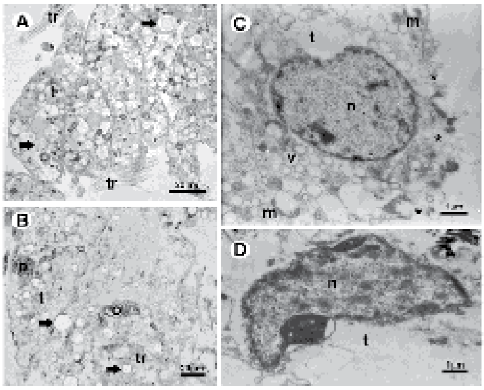

Fig. 5. Perivisceral fat body of the diplopod *R. padbergi* stained with Hematoxylin-Eosin (A; B) and submitted to TEM routine (C; D). Unexposed animal (A); Animal exposed to sewage sludge (B-D). Loss of cell limit and increase of spherocrystal (B); Cytoplasmatic vacuolization and loss of cell membrane integrity (C); Nucleus deformation (D). t= trophocyte; tr = tracheoles; o= oenocyte; m= mitochondria; n= nucleus; v= vacuole; arrows= spherocrystals; *= loss of cell membrane integrity. (Photos: Raphael Bastão de Souza and Larissa Rosa Nogarol)

In diplopods, the fat body, both parietal and perivisceral, is constituted of trophocytes and oenocytes (Fontanetti et al., 2004) and presents intense metabolic activity, such as storage of lipids, uric acid and proteins as well as storage, neutralization and excretion of substances that are not useful (Fontanetti et al., 2006; Hopkin & Head, 1992; Hubert, 1979). In this sense, Souza et al. (2011) exposed diplopods of the species *R. padbergi* in bioassays containing industrial soil contaminated by PAHs and metals (landfarming) in order to analyze histological and histochemical alterations in the perivisceral fat body. The authors concluded that the fat body can be used as a target organ and that the alterations observed, such as loss of integrity of the plasmatic membrane, cytoplasmic disorganization and depletion of energetic reserves can be considered stress biomarkers in this animal. Similar responses were observed in animals exposed to sewage sludge (Abe et al., 2010).

4.2 Genotoxicity biomarkers

The increase in the genotoxic load in the terrestrial ecosystems by the release of chemical products and physical agents can cause impact on the organisms, inducing increase in the frequency of mutations; such effects can lead to a decrease in the size of the population and, eventually, extinction of species and consequently affect the stability of this ecosystem (Majer et al., 2005). In this sense, it became necessary to develop different tests to evaluate the genotoxic potential of soil samples.

Due to the highly conserved structure of the genetic material, it is possible to use a wide variety of species in genotoxicity tests; currently, the most widespread methods for the routine tests are based on the use of indicator bacteria and also basidiomycetes fungi, plants, insects and cultured mammalian cells or even laboratory animals for mutagenicity tests.

According to the literature, the Ames test is the most widely used in genotoxicity evaluations of soils and leachate (Claxton et al., 2010; Wölz et al., 2011). This test, also known as *Salmonella/*microsome, consists, basically, in the employment of strains of the auxotrophic bacteria *Salmonella typhimurium*, i.e., deficient in the synthesis of the aminoacid histidine; the strains of these cells are unable to grow in minimum medium, where the mutagenic compounds are able to restore the synthesis capacity of this aminoacid, thus, the mutagenic expression corresponds to the growth of the colony in a minimum culture medium and it can be easily detected by counting the colonies (Umbuzeiro & Vargas, 2003). However, due to the low sensitivity of the Ames test for heavy metals, more studies should be directed to the development of bioassays with higher organisms (Gatehouse et al., 1990, as cited in Lah et al., 2008).

Meristematic cells of *A. cepa* and *V. faba*, for example, constitute an effective cytogenetic material to analyze chromosome aberrations (figure 6) caused by soil pollution. The use of meristematic cells makes possible the quantification of several morphological and cytogenetic parameters (endpoints), including the morphology and growth of roots and determination of several parameters of cytotoxicity, genotoxicity and mutagenicity. The analysis of the cytotoxicity can be done by determining the mitotic index and cell death. The induction of aberrant metaphases, anaphases and telophases, such as bridges, loss and chromosome stickiness, polyploidy, irregular nuclei and nuclear buds are parameters for the genotoxicity analysis, while the micronuclei and chromosome breaks allow the mutagenicity analysis (Fernandes et al., 2007; Leme & Marin-Morales, 2008; Souza et al., 2009).

Chromosome aberration test concerns the discovery of the mechanisms of action of a particular agent, since the division process is well known. Kovalchuk et al. (1998) state that the chromosome aberrations assay with *A. cepa*, can be used as a tool for quantifying and monitoring genetic alterations in soils radioactively contaminated. Moreover, the chromosome aberration assay in onion was the first of nine plant systems accepted in the Genotoxic Program of the Environmental Protection Agency (USEPA) and is widely used for monitoring residual water. Such sensitivity is attributed by Ma et al. (1995), to the large size of the chromosomes and because they are mostly metacentric. On the other hand, bioassays based on chromosome aberrations, in certain cases, tend to be replaced by less time-consuming techniques, such as the micronucleus assay (MN), which can be performed with mitotic cells in roots (of *V. faba* or *A. cepa*) or meiotic cells, in tetrads of *Tradescantia* (Misik et al., 2011).

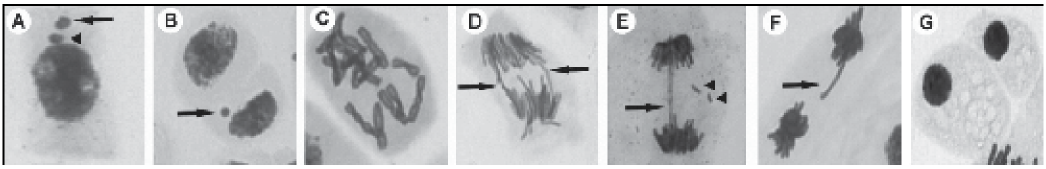

Fig. 6. Alterations observed in *A. cepa* meristematic cells. (A) nuclear bud (arrow head) and micronucleus (arrow); (B) micronucleus (arrow); (C) C-metaphase; (D) anaphase with chromossomal bridge (arrows); (E) telophase with cromossomal bridge (arrow) and chromossomal break (arrows head); (F) telophase with cromossomal delay (arrow); (G) cell death. (Photos: Cintya Aparecida Christofoletti)

The micronucleus test in *Tradescantia* (Trad-MCN) (figure 7) is a sensitive mutagenicity test, of short exposure and simple evaluation, applicable in the species *T. pallida* and in the clones BNL 4430 and KU 20 (Misik et al., 2011). Besides the Trad-MCN, it is possible to evaluate mutations in somatic cells of the staminal hair (Trad-SHM) in young inflorescences of the hybrid clone BNL 4430 (Brookhaven National Laboratory). However, currently, the clone KU 20 (Kyoto University) is more applicable to this technique due to the higher number of inflorescences per cycle. The mutation results in the expression of the recessive allele, which implies in the phenotype of pink colouration. The high rate of pink cells, as well as the loss of reproductive capacity are indicative of mutagenicity (Ma et al., 1996).

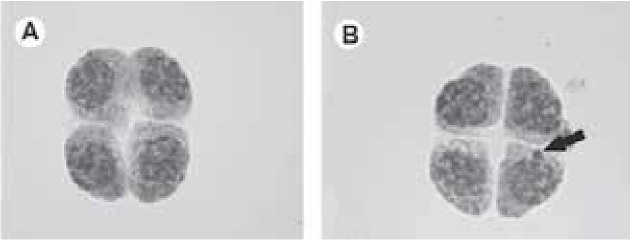

Fig. 7. Micronucleus in polen cells of *Tradescantia* (arrow in B). (Photos: Janaína Pedro-Escher)

4.3 Molecular biomarkers

The use of molecular biomarkers in the environment monitoring represents a significative tool for the evaluation of the contamination in different organisms. Despite morphological markers provide good qualitative evidence of damages caused by certain pollutants, it is known that biochemical alterations resulted from the toxic action of a contaminant are early evidence of negative effects of the exposure, since they precede the onset of visible damages.

Following, it is presented some biochemical biomarkers extensively used in studies of environmental impact, including ecotoxicological analyses of the soil.

4.3.1 Enzymatic antioxidants

The use of enzymatic activity as biomarker is due to the fact that toxic compounds have high affinity for electron pairs found in the aminoacids that form the enzymes (Cogo et al., 2009). One of the main monitored parameters in ecotoxicological analyses of the soil is the concentration of metals. Exposure to metals can intensify the production of reactive oxygen species (ROS), which are normally produced in non-stressed cells and their excess can lead to the oxidative stress and cause harmful effects (Barreiros et al., 2006).

When a cell undergoes oxidative damage, the injuries are minimized in the different organisms by enzymatic and non-enzymatic antioxidants (Freitas et al., 2008). Among the enzymatic antioxidants, some examples would be the superoxide dismutase, catalase, gluthathione reductase and gluthathione -S-transferase (Mishra et al., 2006).

Superoxide dismutase catalyzes the formation of H_2O_2 from O_2. This enzyme was the first discovered among the enzymatic antioxidants and, generally is one of the first to act against damages caused by ROS (Nordberg & Arnér, 2001).

Now, the catalase function is to facilitate the removal of H_2O_2, degrading it in H_2O and O_2. Thus, it reduces the risk of forming the radical hydroxyl from H_2O_2, since this oxygen reactive species is one of the most harmful to the biological systems (Betteridge, 2000; Diplock et al., 1998).

The metabolism of gluthathione is one of the main antioxidant defence mechanisms in the living systems (Valko et al., 2006) and specific metals can induce the synthesis of this compound in different species (Backor et al., 2007). In order to perform its function as oxidant agent, gluthathione must be in its reduced form, reaction catalyzed the enzyme gluathathione reductase (Creissen et al., 1994).

Another important defence system against the increase of free radicals involves the enzyme gluthathione peroxidase, which acts in the removal of hydrogen peroxide and lipid peroxides from the cell (Rover Junior et al., 2001). One of the forms of the gluthathione peroxidase is the gluthatione-S-transferase, one of the most studied detoxicant enzymes in different organisms, since it has an essential role in the cellular response to the stress caused by herbicides in plants. It is considered a detoxification enzyme because it metabolizes a great variety of xenobiotic compounds, catalyzing their conjugation with the reduced molecule of gluthathione and forming substances of low toxicity (Malmezat et al., 2000).

According to Almeida (2003), depending on the type of contaminant and exposure period of the organism to the contaminated environment, the activity of the antioxidant enzymes can be stimulated or inhibited. Generally, the increase of the enzyme activity results from an increase in the production of ROS, which leads to a exacerbated induction of enzymes; now, the decrease can be related to prolonged exposure of the organism to environments highly contaminated, where the production of ROS and the consequent deleterious effects of such production surpasses the defence efforts of the organism.

Several other studies describe alterations in the enzymatic activities of the superoxide dismutase, catalyse, gluthathione reductase and gluthathione peroxidise in different organisms exposed to stress conditions, especially metals, corroborating their use as effective biochemical biomarkers in the evaluation of environmental impacts (Bocchetti et al., 2008; Cogo et al., 2009).

4.3.2 Heat shock proteins

All organisms, from bacteria to mammals, respond to different environmental stress conditions by the synthesis of highly conserved proteins, known as heat shock proteins (HSPs) (Hamer et al., 2004). They are so called because they were first described in cells of *Drosophila melanogaster* during exposure to high temperatures. At the time, it was verified that the exposure of cells to heat produced a new pattern of thickening of chromosomes, which represented specific sites of transcription for the synthesis of proteins. The stress induced the expression of certain genes, which led the cell to produce a certain class of proteins, so-called heat shock proteins. Later, researchers observed that these proteins were expressed in almost all living beings, and not only in response to heat, but also when the cell was exposed to a series of other stressing factors (toxic concentrations of metals, organic pollutants, temperature, osmolarity, hypoxia/anoxia and ultraviolet radiation), and then they began to be called "stress proteins" or "anti-stress proteins" (Meyer & Silva, 1999).

Many toxicants affect the correct conformation and consequently, the function of different proteins. In this condition, where the proteins are found incorrectly folded inside the cell, it is initiated a stress response. The HSPs take action, acting as molecular chaperones, since they bind to other proteins, regulating their conformation, movement through the membrane or organelles and enzymatic activity (Calabrese et al., 2005). Therefore, they avoid incorrect interactions between proteins, helping in their synthesis, folding and degradation (Meyer & Silva, 1999).

According to Bierkens (2000), HSPs are one of the main cellular markers in the evaluation of the toxicity of different compounds and are widely used to monitor ecosystems. Such monitoring has shown high levels of HSPs in the tissues of invertebrates collected in contaminated areas, when compared to those animals existent in uncontaminated environments (Bierkens, 2000; Malaspina & Silva-Zacarin, 2006).

These proteins are found highly conserved in all the living organisms (Burdon, 1986) and can be classified according their molecular weight into four families: HSP90 (90 kDa), HSP70 (70 kDa), HSP60 (60 kDa) and small HSPs. The family HSP70 is one of the most studied and several studies have shown its induction in stress conditions by heavy metals (Köhler et al., 1992, 1996b; Nadeau et al., 2001; Zanger et al., 1996).

Monari et al. (2011) worked with the mollusc species *Chamelea gallina*, observing an increase in the expression of HSP70. The authors affirm that the induction of HSP70 can be considered an adaptation mechanism associated with changes in the environmental parameters.

Silva-Zacarin et al. (2006), using immune-histochemical methods, observed an increase in the levels of the products of the positive reaction to HSP70 in the salivary glands of bees treated with acaricides in comparison with the control group. Moreover, they also verified alterations in the immune-reactivity between the nucleus and cytoplasm according to the acaricide used and the treatment period. According to the authors, the determination and location of HSP70 by immune-histochemistry can be useful to detect cellular responses to chemical stressors.

Köhler et al. (1992) points out the HSP70 in invertebrates of soil as a possible tool in the monitoring of environmental toxicants. In a study conducted by Zanger et al. (1996), the authors exposed adult of the diplopod *Julus scandinavius* to substrates contaminated with different concentrations of cadmium and investigated the expression levels of HSP70. The analyses showed that an increase in the concentration of cadmium in the animals diet resulted in high levels of HSP70.

According to Zanger et al. (1996), the so-called hepatic cells of diplopods can present a strong expression of HSP70 in stress conditions induced by heavy metals. These cells are found disperse through the cells of the "fat body" layer that compose the midgut and one of their functions is to help in the detoxification of the organism (Hopkin & Read, 1992).

In a study carried out by Nadeau et al. (2001), it was investigated the feasibility of using HSP70 as marker of the presence of soil toxicants in the midgut of the earthworm *L. terrestris*. The authors concluded that HSP70 can be efficiently used in the assessment of the toxicity of soils using test organisms exposed under laboratory conditions.

5. Conclusion

Due to the constant release of harmful substances into the terrestrial environment, it is necessary to know their action on the organisms present there, in order to avoid triggering a possible unbalance in the ecosystems. In this sense, we tried to present potential bioindicators and biomarkers for ecotoxicological analyses of the soil. It is important to highlight that the choice of the bioindicator organism is essential to the success of environmental monitoring. Moreover, the combined use of morphological, biochemical and genotoxic methods in the evaluation of injuries in sentinel species is interesting since it provides a more complete understanding of the action of contaminants on organisms exposed, besides providing a greater reliability to the results obtained in the researches. We believe that understanding the importance of the combined use of different methodologies in assessing the toxicity of substrates will be highly beneficial for the future work of researchers in Ecotoxicology.

6. References

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Soil contamination has severely increased over the last decades, mainly due to petroleum hydrocarbons, solvents, pesticides, lead and other heavy metals from industrial wastes and human activities. The critical point regarding contaminated soil monitoring is the intrinsic difficulty in defining fixed monitoring variables and indicators as the establishment of any a priori criterion and threshold for soil quality can be still considered subjective. This book is organized into eight chapters and presents the state-of-the art and new research highlights in the context of contaminated soil monitoring and remediation strategies, including examples from South America, Europe and Asia.

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