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Soil Contamination and Alternatives for Sustainable Development

Edited by Dinora Vázquez-Luna
and María del Carmen Cuevas-Díaz

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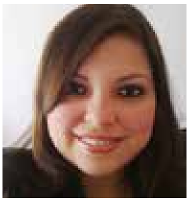

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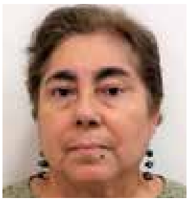

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Contents

Preface

Soil contamination poses an imminent risk to the world, both for its direct and indirect impacts. For this reason, we have chosen six chapters distinguished by their quality and impact from different parts of the world. This book discusses the main effects of soil contamination; as well as some alternatives for sustainable development, with the aim of reducing the harmful effects produced by human activities on the environment. The book is divided into two sections.

In the first section of the book, research by academics with international recognition analyzes the problem of soil contamination and describes the ecotoxicological tests used to assess the quality of the soil. In this section, you will find two cases of soil contamination: one caused by the mining industry, where the biological activity of the soil in a gradient of arsenic and lead concentration was evaluated in Villa de la Paz, San Luis Potosí, Mexico, and another where the flow in unsaturated soils and the transport of herbicides in agricultural areas is described.

In the second section of the book, three sustainable soil alternatives are shown: (1) mitigation of nitrous oxide emissions during nitrification and denitrification processes in agricultural soils using enhanced efficiency fertilizers; (2) management of paddy soils for low greenhouse gas emissions and sustainable rice production in the changing climatic conditions, and (3) biopesticides of neem obtained by enzyme-assisted extraction: an alternative to pest control.

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Section 1

Soil Contamination

Chapter 1

Introductory Chapter: Soil Contamination and Alternatives for Sustainable Development

Dinora Vázquez-Luna and
María del Carmen Cuevas-Díaz

Additional information is available at the end of the chapter

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1. Introduction

Soil degradation and environmental pollution have a great impact on human life, because every year, 2 million people die worldwide due to air pollution [1]; countless numbers of people are exposed unnecessarily to chemicals in the workplace, such as in the external environment [2], because soil, air, and vegetation contain organochlorine pesticides with high carcinogenic risk [3, 4] and heavy metals [5].

Potentially, all previous health problems can be prevented [6]; it has been estimated that in industrialized countries, 20% of the total incidence of diseases can be attributed to environmental factors [7], constituting a big problem for the world population. Toyama, Japan, in 1970, high concentrations of cadmium in rice were found (2.6 and 3.3 g); chronic disease onset was due to rice consumed with 0.36 ppm Cd (155 μg Cd average daily dietary intake), for 58.4 years [8]. Another example was the case of Love Canal in the United States, which is one of the most notorious episodes of soil contamination, where an electrochemical company obtained, in 1942, permission to deposit more than 21,000 tons of its waste; 25 years later, weathering made pollution evident, creating a far-reaching movement for North American environmental policy with lasting effects on public policy [9]. For this reason, there are studies where plants like *Chromolaena odorata* have been found, which is a hyperaccumulating species (100 mg kg^{-1}), and this could be used to eliminate Cd from the soil [10]. Therefore, in this book, the main effects of soil contamination are discussed, as well as some alternatives for sustainable development, with the aim of reducing the harmful effects produced by human activities on the environment.

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2. Soils contaminated by heavy metals

Currently, the agricultural soils suffer from high concentrations of metals, such as As, Cd, Cr, Cu, Pb, Ni, Zn [11, 12] and Hg [13], exceeding the maximum permissible levels and causing a potential risk; industrial soils can be up to 2600 mg kg^{-1} of lead [14], being important to know your inputs and outputs [15]. The countries with the highest cadmium concentrations are Jamaica (200 mg kg^{-1}), India and Pakistan (19–20 mg kg^{-1}), Tunisia, and UK (10–16 mg kg^{-1}) [16]. Other sources of soil contamination are the mining industries and the processing of minerals, which have been found in ascending values: Cd (0.7) → Cu (3.9) → Mn (76) → Fe (79) → Zn (110) → Pb (126) [17].

However, this pollution problem is an important issue for public policies, because the risk assessment must be integrated into the framework of public health and environmental security [18], because concentrations of heavy metals have been found in crops closely related to soil contamination, for example, according to the Chinese environmental quality standard for soil (GB15618–1995), 64.4, 78.9, 67.5, and 94.1% of the soil samples in Dexing, Yangjiazhangzhi, Hongqiling, and Baiyin exceed the maximum allowed concentration of Cd for farmland, respectively [19]. The risk assessment has indicated that priority attention is required due to the carcinogenic risk of Cr, Cd, and As in soil and rice grains, as well as the potential ecological risks [20].

In addition to this, associations have been found between some bacterial genera (*Geodermatophilus* spp., *Rhodovibrio* spp., and *Rubrobacter* spp.) with the presence of heavy metals such as copper, lead, and zinc, respectively [21]. In this regard, **Table 1** shows some examples of heavy metal remediation.

The cost and duration of soil remediation depend on the technique and the site specific, up to \$500 $tons^{-1}$ (\$1500 m^{-3} soil or \$100 m^{-2} soil) and 15 years [30], while it has estimated 20 million hectares of land contaminated by heavy metal.

Type of remediation	**Pollutant**
Extracellular polysaccharides or exopolysaccharides (EPS) are bacterial by-products with various strains that can be bioremediated in contaminated soils [22]	Cu, Co, Pb, Cd, Fe [23]
	Cr, Ni, Cd [24]
Phytoremediation by *Erigeron canadensis* L., *Digitaria ciliaris* (Retz.) Koel., and *Solanum nigrum* L. [25]	Hg, Cd
Biopiles improved soil properties and reduced the solubility of contaminants [26]	Zn, Cu
Bioremediation using *Rhodobacter sphaeroides* [27]	Zn, Cu
Microbial electrochemical system (MES)	Cr [29]
Sodium tripolyphosphate for the synthesis of chitosan nanoparticles from fungal chitosan [28]	Pb, Cu

Table 1. Examples of remediation of soils contaminated by heavy metals.

3. Soils contaminated by hydrocarbons

The global economy based on the oil industry has deteriorated natural resources around the world, with soil being one of the most affected [31]. Total petroleum hydrocarbons (TPH) contain fractions that are toxic to the beneficial organisms of the soil and for humans; this has become an important issue for the development of public policies, food safety, and environmental health because it produces changes in structure and function of soil ecosystems (**Figure 1**) [32].

Oil activities have mainly affected the tropical areas; this pollution has deteriorated soil sustainability, because its toxic effect decreases the ability to support living organisms [33], disrupts the biogeochemical cycles deteriorating ecosystems and altering fertility [34], and reduces quality of soil and agricultural potential [35]. Even hydrocarbons such as benzo[a]pyrene (BaP), belonging to one of the most toxic polycyclic aromatic hydrocarbons (PAHs) in the soil–plant system, are potentially mutagenic and carcinogenic for humans [36]. According to results from researchers in China, they have found between 131,019 m^3 and 146,783 m^3 of soil contaminated with benzo [a] pyrene; it should be mentioned that pollutants are accumulated mainly in the first layer of the site (0–1 meter) [37]. Its main impact on human health is centered on its genotoxic properties (teratogenic, mutagenic, and carcinogenic) (**Figure 1**). The International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) have reported as probable human carcinogens to benzo [a] anthracene, benzo [a] pyrene and dibenzo [a, h] anthracene, benzo [b] fluoranthene, benzo[k]fluoranthene, indene, pyrene and naphthalene; where some researchers have proposed the combination of risk assessments (governmental and computational) [38].

The refining and processing of petroleum can also send numerous chemical compounds to the atmosphere, such as naphthalene, which is considered a dangerous compound in the air

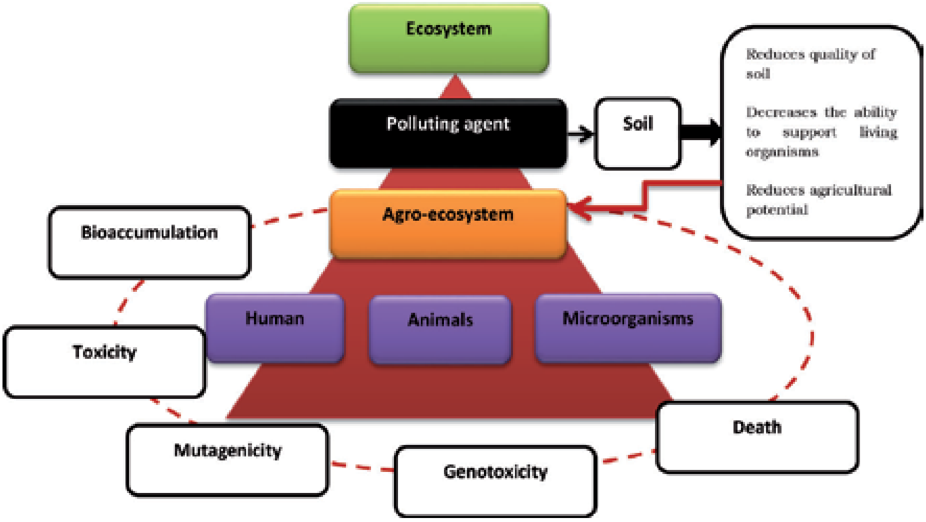

Figure 1. Scheme of the interaction of the pollutant with the agroecosystem.

according to the Environmental Protection Agency of the United States (USEPA), as it can cause irritation in the eyes, skin, and respiratory tract and in high concentrations can destroy red blood cells causing hemolytic anemia [39], besides being considered as a possible carcinogenic in humans (**Figure 2A**).

Another important factor is the contamination associated with heavy metals such as nickel and vanadium which depends on the oil field [40]. Another example is the Amazonia, located in northern Peru, where heavy metal pollution has been found, due to the extraction of hydrocarbons threatening wildlife and local populations that depended on subsistent hunting and fishing [41]; this risk has also been found in La Venta, Tabasco (**Figure 2B**) y en Minatitlán, Veracruz, México (**Figure 2C** and **D**). In this regard, recent studies indicate that the carcinogenic risks of 16 PAH increase with the history of oil extraction; ingestion and dermal contact are the predominant pathways of exposure of the inhabitants to PAH residues in soils [42], representing a risk to the inhabitants, farmers, fishermen, and consumers of these areas.

On the other hand, oil also has various effects on plants due to the changes they produce in the soil; oil can inhibit water retention, causing reduction of germination, emergence, growth, and accumulation of biomass, and cause nutritional imbalance, reduction in pasture production, and, therefore, lower percentage of livestock hectare.

Figure 2. (A) Burning of waste by the petrochemical La Venta, Tabasco, Mexico; (B) sludge in contaminated sites in La Venta, Tabasco, Mexico; (C) petrochemical complex of Minatitlán, Veracruz, México; (D) Santa Alejandrina reservoir adjacent to the Minatitlán petrochemical complex, Veracruz, Mexico.

4. Research proposals

The strategies would be directed toward the integral management of the investigations: (1) the epidemiological study in contaminated areas, (2) the remediation of contaminated soils, and (3) the control of atmospheric emissions.

4.1. Health proposals

we recommend conducting epidemiological studies differentiated by sex, age, and chronic diseases (various types of cancer, asthma, allergic rhinitis, mental problems, depression, etc.); poor air quality, derived from industrial emissions, can influence the increase of diseases, including allergic ones, such as asthma and chronic bronchitis [43], whose pathologies are of high economic cost, in addition, its potential risks as a carcinogenic factor [42].

4.2. Proposals in soils

In the case of contaminated waters and soils, physical, chemical, and biological processes can be used to remove contaminants. However, there is evidence that some of these technologies can cause damage to biota and prevent the recovery of certain habitats [44]. The use of technologies such as bioremediation and phytoremediation are recommended; we propose a mixed process involving several types of remediation, for example, phytoremediation with fibrous root grasses that promote a dense rhizosphere, being the ideal type of vegetation for phytoremediation; it would also be recommended to combine these with biostimulation and bioaugmentation of autochthonous soil remediation bacteria. The vegetal cover besides contributing to the cleaning of contaminated soils can also reduce the transport of contaminants, improving the physical and chemical properties and increasing the microbial activity with better associations of microorganisms with the root and with the toxic compounds in the contaminated soil. Despite this, another problem of great importance is the accumulation of salts, whose effects can greatly harm the growth and development of many plants, so the use of tolerant plants is recommended. It is also necessary to develop sustainable alternatives that reduce pollution and allow the recovery of contaminated resources through remediation processes of soil and water, until pollutants are below the maximum permissible limits.

4.3. Proposals to control atmospheric emissions

In the case of Mexico, it is necessary to follow the guidelines of the Official Mexican Standards on Air Pollution (NOM-085-ECOL-1994). However, it is also necessary to develop sustainable alternatives that reduce greenhouse gas emissions. In addition, to have records and controls of atmospheric emissions, this must be available through the Federal Transparency Law.

5. Conclusions

Anthropogenic activities have caused changes from the global to the local. The health of the inhabitants have deteriorated due to exposure to various pollutants, the incidence of diseases that has increased, new ones that have emerged, and others that have changed their patterns. Therefore, we need to take measures to protect the environment in favor of human health, with synergistic effects on agroecosystem and food safety.

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Chapter 2

Ecotoxicological Tests as a Tool to Assess the Quality of the Soil

Barbara Clasen and Roberta de Moura Lisbôa

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Abstract

Terrestrial ecotoxicology is used to evaluate the effects of substances that, whenever added to the soil, have impact on organisms and help measuring the responses from changes in the lethality, reproduction, development, and behavior of standardized soil organisms. Terrestrial ecotoxicology is a new tool that has been introduced in many countries, including Brazil, and yet little used. However, it is already widely used in Europe, besides being mandatory in research to indicate the toxicity of waste discharged in the soil. The aim of this chapter is to emphasize the importance and need of developing studies focused on the use of terrestrial ecotoxicology as a tool to assess fast and reliable responses from the toxicity of substances incorporated to the soil.

Keywords: contamination, bioindicators, biomarkers, pesticides, soil fauna

1. Introduction

The environment has been exposed to contamination caused by many toxic agents due to human action. Environmental impact, such as soil, surface water, and groundwater contamination, is mainly caused by deactivated industries, contaminant leakage, pesticide use, and inadequate solid and liquid waste disposal. Soil protection and the protection of its inherent communities are acknowledged as the primary aim of environmental policies worldwide [1].

Although the most obvious use of soil lies on plant cultivation for food production, it is also responsible for helping sustainability maintenance. Soil retains water, regulates water resources, and filters and conducts rain water to underground aquifers. It also plays the role

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of recycling raw materials and nutrients, besides being the habitat of a wide variety of organisms such as bacteria, fungi, viruses, nematodes, insects, and worms, among others. The food consumed by living organisms is produced in the soil [2].

The soil, besides being the substance for most man-made food, holds a large amount of pollutants. Chemical and organic fertilizers, pesticides, and other materials applied to the soil often contribute to water and air pollution. Soil is a key component of environmental chemical cycles and an important part of natural resources on Earth. The quality of the soil and climate, which enables productivity, is the most valuable asset of the society [3, 4].

Ecotoxicological tests are internationally acknowledged as complementary tools to chemically analyze soil contamination. However, countries, such as Brazil, do not require the conduction of ecotoxicological tests with soil organisms to evaluate contamination, since it is only based on chemical indicators [5]. The behavior and toxicity of soil elements, or compounds, should not be only assessed based on chemical parameters. Actually, it should be of the utmost importance to include biological parameters in these investigations, since chemical analyses separately applied to the compounds may not show their behavior in the environment [6].

Terrestrial ecotoxicology seeks knowledge about the consequences of chemical-substance discharge in the environment on the organisms living in it. Therefore, it is essential understanding, to which extent, how hazardous the use of chemicals, alone or in mixtures, is, as well as where its effects are observed by monitoring the lethal, morphological, behavioral, physiological, cytogenetic, and biochemical effects on organisms exposed to these pollutants [6, 7].

Among many organisms in soil fauna, which is divided into macro-, meso-, and microfauna, one finds bacteria and fungi and species known as bioindicators. These organisms indicate environmental changes at their early stages and identify several modification types before these changes become worse, besides determining the pollution types capable of affecting a given ecosystem. They also have the ability to help monitoring and making more accurate and less impacting decisions on soil management. There are standardized bioindicator species around the world, such as *Collembola*, earthworms, nematodes, and enchytraeids. The use of bioindicators in monitoring programs helps detecting environmental changes at their early stages or the effectiveness of measures taken to improve environmental quality [7].

2. Soil quality

The quality of the soil is defined by "its ability to function within an ecosystem in a way that it sustains biological productivity, maintains environmental quality, and promotes plant and animal health" [8]. Overall, knowing the soil characteristics involved in the development and sustainability of ecosystems is a useful tool.

Soil quality is assessed through the characteristic of indicators that measure or reflect environmental status or sustainability conditions of ecosystems. Soil quality indicators can be classified as physical, chemical, and biological.

2.1. Physical indicators

The main physical indicators in place are texture, thickness (horizons), soil density, porosity, water, and structure.

Texture refers to particle proportions or to sand, silt, and clay fractions in the soil mass. It is closely related to water retention and transport, soil structure, nutrient content, and organic matter, besides its strong influence on soil erosion processes [9, 10].

Thickness, mainly that of the surface horizon, is essential, since it is the place where the greatest biota activity takes pace. Consequently, the surface horizon is a suitable place for organic matter and nutrient cycling. Soil depth plays a key role given its water storage ability and influence on nutrient supply for plants [10].

The diversity of mineral and organic components, as well as their proportion, determines the density of soil materials. This density changes depending on texture and on soil structural conditions [9, 11].

Porosity is determined by porous space. Part of the soil after the arrangement of solid components is occupied by water and air under natural conditions. Sand retains little water, because its large porous space allows free water drainage from the soil. Clay adsorbs relatively large amounts of water, and its smaller porous space retains water, even with gravity forces [11, 12].

Water amount in the soil depends on climatic factors and on soil texture, structure, and porosity. Water retention capacity reveals the amount of water the soil can store. Hydraulic conductivity is defined by the speed water reaches when it moves in the soil and by its infiltration rates [11, 12].

Soil structure is determined by the geometric arrangement of primary (sand, silt, and clay) and secondary particles (aggregates maintained by cementing agents such as iron, silica, and organic matter) [13].

2.2. Chemical indicators

Many soil functions such as nutrient reservoir, filtration of substances dissolved in water, chemical reaction accelerator, and pollutant immobilizer would not be possible without the presence of chemical components. Organic matter (OM), cation exchange capacity (CEC), hydrogen ionic potential (pH), and chemical elements are often used as chemical indicators of the quality of the soil [14].

Organic matter (OM) is the set of all organic materials in the soil. It is correlated to most soil properties, besides being a key indicator of the quality of the soil [15].

CEC stands out as one of the basic functions of the soil that lies on providing nutrients to plants. This indicator is found on the surface of solid particles in the soil due to electrical charges (Al, Ca, K, Mg, Mn, and Na), although it can be changed by pH and OM [14, 15].

pH is the indicator used to measure soil acidity, and changes in it can affect nutrient availability, microbial populations, and the availability of chemical elements in the soil.

Chemical elements play many roles, but it is possible roughly framing them in nutrients or toxic elements.

Soil nutrients can be divided into two groups:

- Macronutrients (Ca, K, Mg, N, P, S), which are mostly needed by plants.
- Toxic elements, which are considered heavy metals.

Have in mind that the same chemical element, in a certain concentration, can be a nutrient but toxic in another one.

2.3. Biological indicators

Biological indicators, such as microbial biomass, mineralizable nitrogen, microbial respiration, enzyme activity, and metabolic quotient, are fundamental for nutrient cycling and for estimates on soil ability to influence plant growth. In addition, microorganisms provide rapid responses to changes in the environment due to the abundance of metabolic and biochemical activities; therefore, they have great potential to be used as a tool to assess the quality of the soil [16].

The microbial biomass is represented by living components in soil organic matter [17]. This variable controls functions such as the decomposition and accumulation of organic matter or transformations involving mineral nutrients. Biomass provides information about changes in the organic properties of the soil, about changes caused by crops or by vegetation removal, about regeneration after topsoil removal, and about the effects from pollutants such as heavy metals and pesticides [16, 17].

The nitrogen (N) in the soil is the organic compound indirectly available to plants. The N content conversion from its organic form to its mineral one (mineralization) is mainly carried out by bacteria belonging to genus *Rhizobium*, which convert atmospheric nitrogen (N_2) into ammonia (NH_3) through an enzymatic complex. From this point on, ammonia can be incorporated to different forms of organic nitrogen available for plants. From a soil viewpoint, this potential organic N conversion into mineral N (potentially mineralizable nitrogen) has been considered an important factor; therefore, it is an indicator recommended to measure the quality of the soil [8].

Soil respiration reflects the microbial activity and is defined as the biological oxidation of organic matter into carbon dioxide (CO_2), which is conducted by aerobic microorganisms [18]. This indicator occupies a key position in carbon cycles observed in terrestrial ecosystems due to oxidation [19].

Enzymes rule the biological catabolism of organic and mineral components in the soil. This process is closely related to organic matter, physical properties, and microbial activity and biomass; therefore, it is widely used to assess the quality of the soil, since it indicates changes in microbial activity and the presence of pollutants in the soil [14, 20, 21].

The respiratory coefficient represents the relation between carbon in the microbial biomass and total organic carbon (TOC). This coefficient indicates microbial biomass efficiency in using the available carbon for biosynthesis [21, 22].

Besides the aforementioned biological indicators, soil fauna can be used as quality indicator. The biological diversity of the soil is fundamental to maintain soil productive ability; therefore, it is of great importance for the decomposition and mineralization of organic residues, since it favors nutrient availability for plants and even for other individuals. In addition, the soil fauna is sensitive to environmental changes, biological, physical, or chemical. Earthworms (*Oligochaeta*) and isopods (*Isopoda*) are among the organisms most studied as indicators [23].

3. Contamination of soils

Soil contamination is one of the main environmental issues worldwide [1]. The unconscious use of the soil for agricultural activities, for the disposal of waste, chemicals, and industrial waste has been the cause of concern for centuries. Besides huge damages to the environment, a large amount of contaminated soils unviable for agriculture or for construction sites is the consequence of such inappropriate discharge [24]. Agricultural production has led to the increased use of pesticides to control pests and weeds. In addition to active toxic ingredients, many agricultural products contain potentially polluting elements or compounds, such as trace elements and emulsifying surfactants, among others [25, 26]. There has been simultaneous increase in the application of sewage sludge, industrial waste—composed of urban waste—and of agricultural waste for disposal or recycling purposes. These residues have high contents of organic matter and mineral elements capable of improving the chemical, physical, and biological properties of the soil. However, these residues may contain trace elements, pathogens, and many other substances that cause environmental damages [26, 27].

The term "soil contamination" refers to the presence of toxic substances belonging to chemical classes such as inorganic ions (metals), organic solvents, radioactive substances, pharmaceuticals, polycyclic aromatic hydrocarbons (PAHs), and pesticides (herbicide, insecticide, and fungicide) [28].

According to the International Union of Pure and Applied Chemistry (IUPAC), inorganic pollutants are described as "toxic elements." They comprise metal and metalloid elements formerly called "heavy metals." This nomenclature includes elements that, at low concentration, are biologically essential to living organisms. However, when these essential elements are observed at concentrations higher than the required ones or above the recommended limits, they also cause imbalance and exhibit some toxicity.

The displacement of water, soil, and air contaminants, as well as the interface between different compartments, is determined by processes related to the chemical properties of substances in the soil and to environmental compartments. Soil is one of the most complex matrices in the environment due to its heterogeneity. Different soil types present a wide variety of particle sizes and ecophysiological characteristics [14].

Contaminants that reach the lithosphere move by diffusion, that is, water moves through gaps between soil particles. Contaminant displacement speed depends on molecular weight and on the concentration of the contaminant gradient, as well as on soil characteristics such as humidity, clay type, specific area, cation exchange capacity, pH, redox potential, temperature, porosity, and permeability. Soil contamination presents intrinsic characteristics such as cumulative characters and low mobility of pollutants. However, this mobility becomes greater in soil recording relatively low pH or large amounts of sand to the detriment of clay, since these factors make the solubility of toxic elements easier in the environment. Consequently, the soil becomes susceptible to water leach to underground sheets and to other water bodies. Therefore, interactions among the chemical structure of contaminants, soil properties, and entry mode in the environment determine whether a specific substance is persistent and whether it is potentially hazardous to soil compartment [12–15].

The soil must be understood as a living being due to its characteristics, since its biota plays a fundamental role in physical-chemical equilibrium. The soil gets polluted by negative changes in the existing equilibria, which lead to conditions that impair, or makes unfeasible, the life in the assessed area. They also cause environmental damages that can take millennia to be fixed.

4. Terrestrial ecotoxicology

Terrestrial ecotoxicology aims at evaluating the effect of toxic substances on organisms representative of terrestrial ecosystems. This area involves the transportation, distribution, transformation, and final destination given to contaminants found in the terrestrial environment.

Biological processes may be more sensitive to soil changes based on physical and chemical properties, since they suggest that biological indicators could potentially provide early warnings about risks posed to ecosystems [7].

The soil is a heterogeneous mixture of biotic and abiotic factors, besides being inhabited by a wide community of organisms. The fundamental functions of the system depend on its structural and functional integrity. This functionality faces direct impact from changes, and it requires many parameters in order to accomplish a better evaluation [4, 8, 10].

Terrestrial ecotoxicology uses ecotoxicological tests—internationally standardized by the International Organization for Standardization (ISO) and by the Organization for Economic Cooperation and Development (OECD)—which expose bioindicators (animals or plants) to soils contaminated with products or residues of interest in order to qualify and quantify the aforementioned negative effects. Different contamination levels are used in studies in this field [5, 29, 30]; therefore, Inferring the toxicity of the assessed substance and contributing to establish the limits of its use or disposal is possible.

Invertebrate populations in the soil are an appropriate tool to assess the degree of soil disturbance or land use intensification [5] due to human activities [30].

4.1. Ecotoxicological tests

Ecotoxicology is applied through tests that measure the toxicity of substances found in the environment by exposing standard living organisms to them [31].

Ecotoxicological tests allow assessing environmental contamination caused by different pollution sources. These tests have the advantage of covering a wide variety of biological substances available in a sample. They have the inherent ability to detect deleterious effects produced by a toxic agent, or mixture, on living organisms. Thus, these tests allow evaluating how hazardous these substances are.

A large number of ecotoxicological trials have been developed, or improved, due to a wide variety of studied species and ecosystems. Aquatic, sedimentary, and terrestrial systems can be used in tests to verify the degree of contaminations caused by toxic agents and their possible ecological implications [32, 33].

4.1.1. Avoidance test

Among the ecotoxicological tests available, the behavioral test (avoidance test) is a rapid method applied to determine the bioavailability of chemicals or the contaminants in the soil. Although this test is a simple and rapid assay, it has high ecological significance, since avoidance indicates site rejection and population decrease caused by stressor agents such as contamination [34].

The behavioral response results from the level of the organism, which can be defined as the action, reaction, or activation of a given system. This system is subjected to a set of specific conditions that represent the integration of biochemical and physiological processes [35].

Avoidance tests also substantiate the application of other ecotoxicological tests (acute and chronic), since they indicate whether a substance is influencing the physiological and metabolic functions of test organisms.

4.1.2. Acute toxicity test

Acute toxicity tests (mortality test) assess contamination after short-term exposure to high contaminant doses (from 24 h to 14 days). Overall, acute toxicity test results showed that lethality is the main effect of contamination; however, other manifestations such as decreased mobility can be observed [36].

Acute toxicity tests are relatively simple, are inexpensive, and can be applied to a wide variety of organisms. However, they have the disadvantages of not indicating the contaminants responsible for the observed toxicity and the effects of contamination on the dynamics of assessed populations [37].

Results of acute toxicity tests include LC50/LD50 values (concentration/dose causing mortality to 50% of organisms tested) or EC50 (effective concentration or concentration causing an effect, other than mortality, that is, immobility to exposed organisms) [38].

4.1.3. Chronic toxicity test

Chronic toxicity (reproduction test) tests are closely linked to results of acute toxicity tests, since sublethal concentrations are calculated based on LC50. These tests evaluate the effects of lower contaminant concentrations for long exposure time. The observed effects are sublethal and emerge when the toxic agent concentration the organisms are exposed to allows the survival of these organisms. However, these concentrations affect one, or more, biological function of these organisms, and it influences reproduction and egg development and growth [40].

Chronic toxicity tests may be long and laborious; sometimes they range from 4 to 7 weeks, and these factors are the main disadvantage of applying this test type [37]. However, they are the most sensitive tests, which are considered the most relevant to predict the impacts on ecosystems, since they demonstrate the dynamics of populations over time [41].

Results of chronic toxicity tests can be expressed in CENO (concentration of observed effect, the highest concentration of the tested sample, which does not cause deleterious effect) or in CEO (concentration of observed effect, the effect of the lowest concentration on the body) [39].

The use of ecotoxicological tests aims at integrating different information to plan, to make decisions about public health, to take environmental control measures, to define remediation techniques, and to hierarchize the areas to be prioritized by environmental recovery programs.

4.2. Biomarkers and bioindicators

Biomarkers are tools used to measure the effects of the exposure to toxic compounds and its potential impact on living organisms, including humans. Bioindicators are changes resulting from the presence of xenobiotics in components and in cell biochemical processes, structures, or functions measurable in a system or sample [42].

Indicator organisms are used in the testes due to their characteristics, since they present very short ecological tolerance limit. Therefore, they present some physiological, morphological, and/or behavioral changes when they are exposed to certain contaminants [32].

The low cost of bioindicators is their advantage in comparison to conventional methods used to evaluate environmental quality. They can also be used in the cumulative evaluation of events observed within a given period of time to recover an environmental history that cannot be detected through other methods [23, 31, 32].

The soil hosts the greatest diversity of organisms on the planet; however, these organisms can be affected by the substances deposited in it [16, 34]. Hundreds of thousands of invertebrate species contribute to decomposition processes by crushing organic matter, improving the mineralization process and, consequently, the nutrient cycling and energy flow in ecosystems [16, 43].

Ecotoxicological tests have been widely used to evaluate the environmental impact of several pollution sources such as hydrocarbons [34, 36], toxic sludge from the ceramic [44] and textile industries [45], domestic effluent [46, 47], persistent organic pollutants (POPs) [48], and bovine manure [49].

However, ecotoxicological studies conducted in terrestrial environments remain relatively incipient in comparison to the ones involving aquatic environments. Most studies in this field are concentrated in Northern countries, and this evidence highlights the need of verifying the possible impacts of this situation on the soil of different regions of the world [27, 30].

Edaphic organisms are among the indicators used to measure the quality of the soil, given their importance to the decomposition, cycling, and regularization of nutrients in biological systems [23].

Earthworms (*Oligochaeta*) are among the most studied edaphic organisms to be used as bioindicators. These organisms are bigger than 2 mm (soil macrofauna) and have direct influence on soil functioning [50].

4.3. *Eisenia* sp. (*Oligochaeta, Lumbricidae*)

Species belonging to genus *Eisenia* sp. (*Oligochaeta*, *Lumbricidae*), commonly known as Californian or red worms of California [16, 23], stand out among terrestrial fauna species used in toxicity tests. *Eisenia fetida* and *Eisenia andrei* naturally live in the topsoil, animal manure, and compost materials. They are soil organisms that participate in soil aggregation processes and in the decomposition of plant residues, thus maintaining soil fertility and the quality of agricultural and natural ecosystems [40].

Earthworms are exposed to contaminants through skin absorption and intake. A yellow fetid substance acts in defense of the earthworms when they are threatened by pores in the upper surface of the body—this substance keeps predators away from them [51–53].

Oligochaetes are often used to evaluate soil contamination because they ingest a large amount of soil and demonstrate the ability of accumulating pollutants [54–56]. They crush organic matter and produce excellent-quality humus, fact that facilitates water and air entry into the soil and helps combating erosion and recovering degraded soils [57].

According to [58], earthworms are widely used given their suitability for the bioavailability evaluation of many soil chemicals due to the following factors:

- Earthworms live on the ground and are in constant contact with the soil.
- They live in contaminated sites, and it allows the validation of chemical availability in the field.
- They are found in a wide variety of soil horizons.
- The epidermis of the worms is vascularized, but it has no cuticle, and it allows absorbing the contaminants straight from the soil.
- Earthworms ingest specific fractions of soil, and it provides the means for contaminant uptake.
- They present high body mass, and this factor helps determining the concentration of contaminants in the assessed individuals.
- Their physiology and the metabolism of metals in their organisms are well known.
- Availability of standard test protocols.
- Some species, such as *E. fetida*, are easy to cultivate, can be kept in laboratory under controlled conditions, and are tolerant to different soil types.

Pollutants in soils have direct contact with clay and with organic materials highly capable of binding to chemical compounds and substances [14, 15, 19]. Earthworms get in contact with pollutants when they excavate and ingest contaminated soil or litter; they also absorb contaminants from the soil solution that passes through the cuticle. So earthworms can poison themselves with these pollutants; they can die or survive by incorporating or even bioaccumulating these pollutants in their tissues. This ability can be a threat to their predators, since earthworms are an important link in the terrestrial trophic chain; they are on the diet of several animal species [59–63].

Procedures followed in ecotoxicological tests conducted with earthworms are established by national and international standards. There are internationally acknowledged standards proposed by International Organization for Standardization (ISO) and Organization for Economic Cooperation and Development (OECD). However, some countries have their own standards. For example, in Brazil, the NBR 15537 (2014) and NBR ISO 17512-1 (2012) regulate the application of test with earthworm conducted to evaluate acute toxicity and behavior, respectively.

Table 1 shows a selection of standardized toxicity test protocols developed by acknowledged institutions involving worms currently available for researchers.

Many studies focus on artificially contaminated soil; they use to add a single substance to the assessed soil. In addition, these studies are conducted under conditions that do not properly reflect the reality in the field.

4.3.1. Ecotoxicological studies already carried out with earthworms belonging to genus Eisenia sp.

Table 2 shows a summary of results recorded by some authors in studies conducted with *Eisenia* sp., based on behavioral tests (avoidance).

Table 3 summarizes the results recorded by some authors in studies with *Eisenia* sp. based on ecotoxicological trials conducted to measure lethality (acute test) and reproduction (chronic test).

Laboratory soil toxicity tests have advanced in recent years given the introduction of soil invertebrates in them; consequently, the adoption of avoidance toxicity tests has increased [34].

Test	Duration (days)	Species	Standard
Lethality	14	*E. fetida/E. andrei*	OECD 2017 (1984) ISO 11268 (2012)
Reproduction	28 + 28[1]	*E. fetida/E. andrei*	ISO 11268-2 (2012) OECD 222 (2004)
Avoidance	2	*E. fetida/E. andrei*	ISO 17512-1 (2012)
Bioaccumulation	Until 21 + 21[2]	*E. fetida/E. andrei*	OECD 317 (2010)
	7 to 28	*E. fetida*	ASTM E 1676-12 (2010)

[1] 28 days for cocoon production + 28 days for juvenile hatching.

[2] First stage, up to 21 days for the absorption of the test substance; second stage, 21 days for elimination.

Table 1. Standardized toxicity tests using earthworms as test organisms.

Author	Results
[37]	They exposed *E. andrei* specimens to different substances (copper sulfate, pesticides, dimethoate, carbendazim, benomyl) in soil collected in a deactivated mine Results: avoidance—copper sulfate, 320 mg/kg, earthworms in the assessed mine showed no preference between the contaminated soil and the control
[36]	They evaluated the behavior of *E. fetida* specimens in samples contaminated with hydrocarbons Results: 96% of individuals fled the section containing the contaminated sample
[64]	They evaluated the behavior of *E. andrei* specimens exposed to three sludge types: treated domestic effluent, treated canning industry effluent, and the sludge from a galvanic treatment plant Results: Organisms presented greater attraction to sludge from the sewage treatment plant and treated canning industry effluent due to the higher organic matter concentrations in them. There was avoidance reaction at low concentrations of sludge from the galvanic industry, mainly due to the high content of chromium in it
[65]	The authors analyzed soil and sludge mixtures from three sewage treatment plants in Germany. Results: The sludge was toxic to the test organisms (*E. fetida*) at concentration 9 g sludge/kg soil (EC50, 13.4 g sludge/kg soil). About 100% contaminated soil evasion was observed at 45 g/kg
[66]	Assessed the effects of herbicides Diuron and fluazifop-p-butyl on *E. andrei* Results: The avoidance behavior in the soil evidenced that both herbicides caused significant avoidance response
[67]	Studied the avoidance behavior to silver forms (nanomaterials (NMs)) at four time points (24, 48, 72, and 96 h) Results: $AgNO_3$ and NM300K induced avoidance response in *E. fetida* at concentrations 10 and 100 times lower than the concentrations required for AgNM-PVP coated and AgNM-non-coated nanomaterials
[34]	The authors investigated the toxicity of a binary petroleum hydrocarbon (PHC) mixture to the avoidance response of five soil invertebrate species (*E. fetida, Enchytraeus crypticus, Folsomia candida, Oppia nitens*, and *Hypoaspis aculeifer*) Results: The avoidance of invertebrates in PHC-contaminated soil was similar in growth measurements applied to plant species sensitive to PHC-contaminated soil

Table 2. List of results recorded by some authors in a study focused behavioral tests conducted with earthworms.

There is lack of data about the effects of herbicides on earthworms because they are often seen as low or nontoxic. [40] investigated whether the widely used commercial formulations of glyphosate (GLF), tembotrione (TBT), and nicosulfuron (NCS), which are applied at three environmentally relevant concentrations, have adverse effects on different biomarkers and on the reproduction of the epigeic earthworm species *Dendrobaena veneta.* The tested herbicides did not have significant effect on reproduction success. GLF induced the acetylcholinesterase (AChE) activity after 7 days and NCS, after 28 days, whereas TBT caused up to 47% inhibition after 7 days. Only TBT caused significant change in catalase (CAT) after 7 days of exposure. Malondialdehyde concentrations (MDA) increased after NCS exposure (at any exposure period), but it only happened in GLF and in TBT after 7 and 28 days, respectively. The activity of measured biomarkers changed depending on the applied herbicide and on exposure time; it also suggested that oxidative stress plays an important role in the toxicity of the tested herbicides.

Author	Results
[63]	Eight soil samples were collected from seven abandoned mines in the United Kingdom Results: Cocoon production and hatching rates showed that they were more sensitive to survival conditions or to weight changes. The most toxic soil presented low organic carbon, and sandy soil presented intermediate metal concentrations (7150–13,100 mg Pb/kg, 2970–53,400 mg Zn/kg)
[64]	Evaluated the behavior of *Eisenia andrei* specimens exposed to three sludge types: treated sewage, treated effluent from an olive plant, and sludge from a galvanic treatment plant Results: Mixtures containing domestic sewage and sludge from the olive industry increased the production of juvenile organisms and stimulated the growth of earthworms. However, the mixture with sludge from the galvanic industry caused significant decrease in the production of juveniles
[68]	The authors carried out an ecotoxicological evaluation with oligochaetes (*E. andrei*) living in soil treated with sewage Results: The applied doses did not present significant mortality under acute exposure, but they caused lethal effects under chronic exposure. In addition, the test organisms bioaccumulated metals (Cu, Hg, Pb, Zn) because they are bioavailable and due to the role played by Cu and Zn in the metabolism and physiology of oligochaetes
[48]	Evaluated the ability of *E. andrei* species in bioaccumulate hexachlorobenzene, which is a persistent organic pollutant (POP) Results: Annelids accumulated hexachlorobenzene in their tissues, and this process may result in biomagnification along food chains
[49]	Evaluated the toxicity of bovine clearance residues on the survival rate of *E. fetida* Results: They found 100% lethality in individuals subjected to the treatment with waste at concentrations 84.12 mg/kg Zn and 323.11 mg/kg Cu. The increased Cu and Zn concentrations reduced the number of cocoons
[47]	The authors carried out acute and chronic toxicity tests with *E. fetida* specimens in sanitary effluent sludge. Results: The sewage sludge showed no acute toxicity effect. The reproduction test presented the deleterious effect of it on the reproduction of worms, since there was the absence of cocoons and of young organisms after the 14th day of testing
[69]	Evaluated dredged sediments in Guabanara Bay, Rio de Janeiro, Brazil, through toxicity tests. The bay has been highly impacted by the disposal of liquid domestic and industrial waste Results: *E. andrei* reproduction was sensitive to dilute sediment samples presenting EC 20 = 1.26%, EC 50 = 2.94%. In addition, surviving earthworms showed visible morphological damage in their epidermis
[29]	The toxicity of five pesticides (trichlorfon, dimethoate, carbendazim, tebuconazole, and prochloraz) typically used in rice farming was evaluated through the mortality and body weight of *E. fetida* specimens 7 and 14 days after the beginning of the experiment. Results: The insecticide dimethoate showed moderate acute toxicity, whereas the other tested pesticides showed low toxicity potential. However, weight loss was identified as a sensitive endpoint with the use of these pesticides
[70]	Evaluated the chronic toxicity of the abovementioned six neonicotinoids (NEOs) to *E. fetida*—its cocoon production, hatchability, cocoon weight, and adult weight were affected in the test Results: Cocoon production and hatchability were more sensitive than cocoon weight and adult weight. The reproduction of earthworms was significantly reduced at 56 days half-maximal effective hatchability concentrations (EC50) 0.37, 0.74, 1.30, 3.57, 1.20, and 0.70 mg/kg (acetamiprid, dinotefuran, clothianidin, thiacloprid, nitenpyram, imidacloprid), respectively. Most tested NEOs were highly toxic to *E. fetida*

Table 3. List of results recorded by some authors in a study focused on lethality and reproduction conducted with earthworms.

Ref. [68] evaluated bioaccumulation in organisms of oligochaetes exposed to soil treated with sewage sludge and found that earthworms absorbed Zn and Cu metals in their tissues. In addition, the chronic assay caused lethal effects, as well as the absence of cocoons, at the end of the bioaccumulation stage. This outcome evidences that the toxics found in sewage sludge were able to affect the reproduction of oligochaetes.

There was significant copper (Cu) uptake increase by earthworm (*Eisenia fetida*) when there was combined benzotriazole (BTR) pollution in the soil [26]. According these authors, water and soil environments contaminated with triazole can form complexes of metal ions and, therefore, affect the bioavailability and toxicity of some heavy metals.

Ref. [71] verified the toxicity rates in *Eisenia andrei* species exposed to different aluminum concentrations and found that the metal was toxic (457 mg Al/kg) at lower pH values (3.24). The growth and production of cocoons reduced at this pH.

Ref. [72] evaluated the toxicity of different aluminum concentrations to *E. fetida*. Their results indicated that its survival was not affected until 1444 mg Al/kg, but it reduced by 85% at concentration 2222 mg Al/kg.

Some factors influence test results, and they should be carefully evaluated based on their interference.

According to [73], soil characteristics in extremely sandy soils influence the results of ecotoxicological tests. Earthworms tolerate a wide range of pH (from 4 to 9), but they prefer neutral to slightly acidic pH conditions (from 5 to 7). Ref. [74] studied the reproduction of *E. andrei* in artificial soil and found reduced number of juveniles at pH values 4, 6.5, 7.5, and 8, as well as optimum cocoon production at pH between 5 and 6.

Earthworms prefer soil with high levels of organic matter. According to studies, earthworms strongly tend to avoid soil with low organic matter content [75]. In addition, several authors [76–78] indicate that organic matter forms stable complexes with metals by reducing their bioavailability and geochemical mobility.

Ref. [64, 69] point out that oligochaetes recognize the organic matter in domestic sewage and in sediments as real food sources.

Ref. [71] sought to analyze the reproduction of earthworms and the production of cocoons of organisms exposed to different Al concentrations and pH. Based on the recorded results, low Al concentrations and higher pH values reduced the production of cocoons. These authors suggest that increased oligochaete biomass may have implications in the reproduction of these animals, since organisms use much of their metabolism energy to increase their biomass; thus they reduce their reproduction and cocoon production rates.

Ref. [69] found biomass loss in test organisms subjected to the treatment with 20% dredged sediment from a pond receptor of domestic and industrial effluent. Weight loss likely resulted from the fact that energy reserves are mobilized to allow detoxification processes that reduce the energy allocated for growth purposes.

The greater attraction of organisms to soil with high organic matter content does not exclude toxicity likelihood, and this observation evidences the need of carrying out a test at chronic level.

Thus, it is essential knowing soil characteristics, since they can influence stress factors in organisms, other than the ones related to the contaminant that influence test results.

Recently, lethality and reproduction are not only analyzed through toxicity trials. Biochemical responses and DNA damage are complementary approaches to standard toxicity tests, since they provide more information about body responses to stress in mixtures [79].

Ref. [79] evaluated the activity of superoxide dismutase, acetylcholinesterase, cellulase, and DNA damages in *E. fetida* living in soil contaminated with heavy metals. Based on their results, there was lower sensitivity to superoxide dismutase enzyme activity, whereas dismutase, acetylcholinesterase, and DNA damage were more sensitive.

Ref. [80] evaluated changes in superoxide dismutase, catalase, peroxidase, cellulase, and malondialdehyde in *E. fetida* exposed to the insecticide imidacloprid, which is widely used in agriculture. Results showed that all evaluated enzymes recorded changes in their activity as the imidacloprid concentration increases (>0.66 mg/kg).

The biochemical and genetic toxicity of dinotefuran on *Eisenia fetida* were evaluated at 0, 0.1, 0.5, 1.0, and 2.0 mg/kg in a study conducted by [81]. Dinotefuran induced excessive reactive oxygen species (ROS) generation at 1.0 and 2.0 mg/kg, and it resulted in significant changes in the activity of antioxidant enzymes and on the functional gene expression. Moreover, lipids, proteins, and nucleic acids were oxidized and damaged by the excess of ROS induced by dinotefuran—this process results in serious destruction of cell structure and function.

Research such as those conducted by [79, 80] are promising, but further research is needed in order to explain certain mechanisms, given the complexity of some enzymes, as well as DNA damage and its possible consequences on organisms.

5. Conclusions

Natural soil resources must be interpreted and studied by taking into account all their compartments, since they act together in different soil functions.

Contaminated soils have been largely monitored through physical-chemical analyses. However, soil quality assessments and synergistic effects caused by contaminants are not taken into account. Ecotoxicological tests are critical for the evaluation of potential environmental risks posed by these contaminants.

It is important carrying out tests by using organisms that represent terrestrial ecosystems in toxicological evaluations. Toxicity tests are tools that help the management of contaminated areas. They indicate the viability of natural environmental recovery, since organisms in the field degrade and mineralize substances in contaminated environments.

It is recommended to extend the present study to other organisms, such as microcrustaceans, living in different habitat types (sediments and water) in order to cover different trophic levels and to assess whether there is, or not, toxicity transfer between food chain levels.

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Chapter 3

Evaluation of the Biological Activity of Soil in a Gradient Concentration of Arsenic and Lead in Villa de la Paz, San Luis Potosi, Mexico

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Abstract

The mining industry has generated different sources of pollution and effects in human and ecosystem health. The objective of this study was to evaluate the effect in gradient of the biological activity of the soil derived from the contamination by lead and arsenic. A linear transect was conducted from the tailings in Villa de la Paz to a reference site 10 km away against wind direction and runoff. The parameters that were analyzed in the soil samples included As, Pb, pH, organic matter, electrical conductivity, soil respiration, and soil enzymes. As concentrations ranged from 4.7 to 463.2 mg/kg, while those of lead ranged from 171.7 to 2319.0 mg/kg. The changes in the physicochemical parameters and in the biological activity of the soil were stronger within the first 100 m of distance from the tailings. The pattern of inhibition of biological activity was: urease>β-glucosidase>arylsulfatase> dehydrogenase>phosphatase>respiration. Strong negative relationships were observed among biological activities and arsenic (from 86.5 to 96%). Metals and covariables jointly explain the 89.2% of variability of the effect in the biological activities. This study provides a field baseline that could be part of a long-term monitoring and remediation program.

Keywords: pollution gradient, soil enzymes, soil respiration, soil pollution, tailings

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1. Introduction

The soil is the upper layer of the solid surface of the planet, formed by weathering rocks, where plants can or may be rooted in and which is an ecological environment for certain types of living beings [1]. Soil is a living, dynamic and non-renewable resource, and its condition and functioning are vital for food production, and for the maintenance of local, regional and global environmental quality. The processes of decomposition and respiration that take place in it play a key role in maintaining the balance between the production and consumption of CO_2 in the biosphere [2]. The soil is composed of mineral particles of variable sizes, organic matter and numerous species of morphologically and physiologically distinct microorganisms. Currently, there is a growing concern about the sustainable use of land in terms of agriculture, environmental quality and human health, as well as its degradation. Soil health can be considered as "*the continuous ability of the soil to function as a living system, within the limits of the ecosystem and land use, to sustain biological productivity, to promote the quality of air and water environments, and maintain the health of plants, animals and humans*" [3].

The biological activity of the soil can be reflected in processes such as respiration and enzymatic activity [4]. Enzymatic activity in the soil is mainly of microbial origin that is derived from the intracellular enzymes associated with the cells or the physiological processes of the organisms. Enzymes are direct mediators for the catabolism of the biological components of the soil (organic and mineral). Therefore, these catalysts provide a meaningful evaluation of reaction rates of important processes that occur in the soil [5]. Breathing and activities of soil enzymes can be used as direct measures of microbial activity, soil productivity, and the effects of inhibition due to the presence of contaminants [6]. Microorganisms can react quickly to changes in the environment with alterations in metabolic activity, biomass and the structure of the community [7]. Due to the above, enzymes and respiration have been proposed as indicators for the monitoring of soil quality and the variation of microbial activity [8]. Respiration and enzymatic activity can be influenced in different ways by heavy metals. Dehydrogenase is present only in living organisms and represents active biomass, and it carries out a wide range of oxidation activities that are responsible for the degradation of soil organic matter [9]. Phosphatase is an extracellular enzyme; it hydrolyzes organic phosphorus compounds in different inorganic forms, which can be assimilated by plants [10]. Urease participates in the hydrolysis of urea into carbon dioxide and ammonia, which originates from microbes and shows extracellular activity [11]. Phosphatases are a group of enzymes that catalyze the hydrolysis of phosphoric acid esters and anhydrides. In this group, we can find the mono-ester-phosphate hydrolases, in which acid and alkaline phosphatase are found, which are nonspecific enzymes that catalyze the hydrolysis of glycerol phosphate [3]. Glycosidases (β-glucosidase and β-galactosidase) have the ability to intervene in the biogeochemical cycle of carbon and act on glycosides by catalyzing the release of sugars [12]. Overall, a general pattern of inhibition of enzyme activity and respiration due to heavy metal exposure in the soil has been observed in some studies [13].

Mining is considered one of the oldest and most fundamental activities of mankind [14]. Mexico has been characterized as a mining power. At present, it occupies the first places in the production of arsenic, cadmium, lead, mercury, fluorite, barium, among others, worldwide.

This large production is due to the fact that Mexican mining areas are located in large zones of the national territory. Thus, mining has become an important economic sector for states such as Guanajuato, Sonora, Chihuahua, Durango, Zacatecas, San Luis Potosi and Hidalgo [15]. Mining is distinguished by its different effects, magnitudes and environmental impacts, which can be of two types: the first includes the modification of the landscape, habitat, hydrological regime, changes in topography, among others; and the second involves pollution from the inadequate management of generated waste (e.g. tailings and tails) in the mineral extraction and processing stage, this type of waste usually comes with high concentrations of metals and metalloids [16]. The tailings are generated in the process of concentration of lead minerals, silver, zinc and copper; it usually contains residual metal sulfides such as pyrite, pyrrhotite, galena, sphalerite, chalcopyrite and arsenopyrite, which are sources of potentially toxic elements (PTE) such as arsenic (As), cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), iron (Fe), and so on, which in high concentrations have toxic effects and are considered environmental pollutants capable of altering ecosystems [17–20]; furthermore, these elements are not biodegradable, they can bioaccumulate and some of them biomagnify [21].

The objective of this study was to evaluate the effects of As and Pb on the biological activity of soil in a distance gradient to the mining waste in Villa de la Paz, Matehuala, considering respiration and enzymatic activity as indicators of ecological integrity and soil health.

2. Materials and methods

2.1. Study site

The mining district of Santa María de la Paz is located in the municipalities of Villa de la Paz and Matehuala in the state of San Luis Potosí (**Figure 1**). In this district, there is a skarn deposit of Pb-Zn-Ag (Cu-Au) (metamorphic rocks constituted by silicates of Ca, Fe and Mg derived from a protolith of limestones and dolomites in which large amounts of Si, Al, Fe and Mg have been introduced), which has been exploited continuously for a little more than two centuries. The municipal seat of Villa de la Paz is located on the following coordinates: 100°42′47“ west longitude and 23°40′31″ north latitude, at an altitude of 1837 m above sea level, with an extension of 131.33 km^2 and a population of 5350 inhabitants [22]. The municipality has a temperate dry climate. The annual average temperature fluctuates around 18°C; the hottest month is June with 28°C and the coldest month is January with 3°C. Rainfall is 486 mm per year [23]. In the region, there are no important fluvial currents, only intermittent streams, which are formed in the mountains and transport water only during torrential rain events, without any economic or social use are located, but the water they carry is captured on different boards that are used to provide cattle with a trough service and to a lesser extent for land irrigation [24]. Lithosol, xerosol and rendzina soils with petrocalcic and calcareous phases predominate in a remarkable way [25] (CEFIM, 2012). The predominant vegetation types are thorny desert scrub, microphyll, nopal, izotal, cardonal and grassland scrub. Fauna is characterized by species such as hare, rattlesnake, wild cat, wild birds and field mice. The productive activities of the area are mainly: (1) agricultural (tuna, pastures and meadows groomed, corn and beans), (2) livestock with activities of production of bovine meat (8 tons/year), swine (9 tons/year), sheep (1 ton/year), goats (19 tons/year) and production of bovine milk (18,000 L/year) and goat milk (23,000 L/year) [26] and (3) mining industry, which occupies 38.66% of the total area of the municipal capital, therefore the site is a typical mining area [23].

During the time of operation of the mining activities in the area, contamination by heavy metals and arsenic has been generated in soil, water and sediment [24]. In recent years, several studies have been conducted in the area in which the presence of high concentrations of heavy metals and As in water, air and sediment has been demonstrated [24, 27–29]. In the study by Razo et al. [24], tailings, dams and slag deposits were identified as the main sources of pollution and dispersion of particles due to wind (**Figure 2**). On the other hand, it has been proven that there are health risks to human and ecological populations in the area, due to exposure to heavy metals and As [30–36].

2.2. Soil sampling

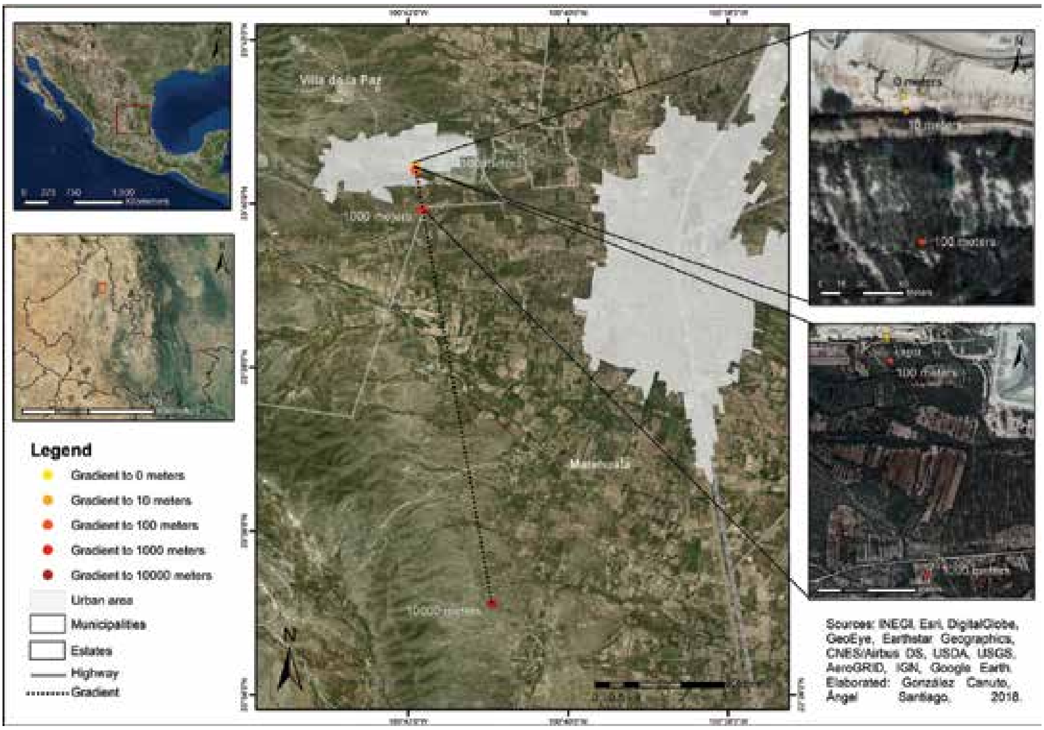
Twenty-five composite samples of surface soil were collected in a distance gradient located from the source of contamination (tailings); the gradient was established on a linear path at distances of 0, 10, 100, 1000 and 10,000 m (**Figure 1**); for the location of the route, the predominant direction of the winds and the intermittent water runoff in the area were considered. For each distance, five subsamples of 10 cm^3 of soil collected in an area of 1 m^2 were gathered. Sampling was carried out on October 2016. The samples were homogenized, sieved (2 mm), placed in 50 mL sterile conical tubes and placed at 4°C for transport to the laboratory, where they were stored for 7 days at −20°C for their analysis.

2.3. Analysis of arsenic and lead in soil

The samples were dried in an oven (at 30°C) until they reached a constant weight (from 24 to 36 h), and moisture percentage was determined. Samples (0.5 g of soil) were placed in teflon cups with 10 mL of 25% ultrapure HNO_3. Acid digestion was performed in a closed system with a microwave oven (MDS-2000-CEM) at 100 W of power and 80 psi of pressure for 1 h with a TAP of 30 min. The digests were filtered and calibrated to 10 mL. The quantification was performed by atomic absorption spectrophotometry with a hydride generator in the case of As (PerkinElmer AAnalist 100 Flame AA) and a graphite furnace for Pb (PerkinElmer PinAAcle 900 T). In order to calculate the concentrations, calibration curves were made with standards, and as a quality control, blanks and reference materials were used (Montana soil l-NIST-2710a). The percentages of recovery were from 90 to 110%. The samples were processed in duplicate, and the results expressed in mg/kg.

2.4. Physicochemical parameters

pH was determined using the water measurement method [37] in a 1:2 ratio. About 6 mL of deionized water were added to a sample of 3 g of soil and then were kept under agitation for three cycles of 5 min at 800 rpm. The electrical conductivity was measured using the method mentioned in [37] at a soil-water ratio of 1:5. About 15 mL of deionized water were added to a sample of 3 g of soil and then were kept under agitation for three cycles of 5 min at 800 rpm. The measurements of pH and electrical conductivity were calculated in the



Sources: INEGI, Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, Google Earth. Elaborated: González Canuto, Ángel Santiago, 2018.

Figure 1. Study area and location of sampling sites.

Figure 2. Mine tailing deposits and acid drainage.

supernatant after the sample had been resting for 15 min in a benchtop multiparameter meter (HANNA-HI2550). Organic matter was determined using the wet digestion method [38]. In addition, 2.5 ml of $Na_2Cr_2O_7$ solution (0.5 M) and 5 mL of concentrated H_2SO_4 were added to a sample of 0.250 g of soil. The mixture was allowed to stand for 5 min after which 17.5 mL of deionized water were added, and it was stirred at 150 rpm for 30 min. The quantification was performed after 24 h using a UV–visible spectrophotometer (Biomate 3 s Thermo Fisher Scientific) at 600 nm in 2 mL of supernatant. All samples were made in duplicate.

2.5. Respiration and enzyme assays

Soil respiration was determined using the MicroResp method [39], which consists of filling a 96-well plate microplate (1.2 mL per well) with soil, a carbon source (25 μL of glucose at 25%) and water (375 μL). The plate was subsequently sealed (silicone rubber gasket with interconnection holes) with a CO_2 colorimetric detection trap consisting of a microplate (300 μL per well) with 150 μL of agar enriched with KCl (0.15 mol L^{-1}) and cresol red dye (32.7 μmol L^{-1}). Incubation was carried out at 25°C for 6 h in dark conditions. The absorbance in the detection plate was measured at 570 nm. The amount of CO_2 released was calculated using a calibration curve. The results were expressed in μg C-CO_2 g^{-1} soil h^{-1}.

Arylsulfatase was quantified according to the method of Tabatabai and Bremner [40]. The method is based on the hydrolytic capacity of the enzyme on an artificial substrate (p-nitrophenyl sulfate) whose product p-nitrophenol is evaluated spectrophotometrically at a wavelength of 420 nm. The activity of arylsulfatase was expressed in μg of p-nitrophenol g^{-1} h^{-1}.

The β-glucosidase was determined according to the method of [41]. The method is based on the quantification of p-nitrophenol obtained by the action of glycosides after incubation of the soil with the substrate β-D-glucopyranoside in medium at pH 6. The incubation was carried out at 37°C for 1 h; the released p-nitrophenol was removed by filtration after having added $CaCl_2$ and adjusted with THAM buffer at pH 12. The absorbance was recorded at 405 nm. The activity of β-glucosidase was expressed in μg of p-nitrophenol g^{-1} h^{-1}.

The activity of the enzyme dehydrogenase was determined by the method of [42–44]. The method is based on the measurement of iodonitrotetrazolium formazan (INTF) produced by the reduction of 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium (INT) in soil incubated with INT in buffered medium under dark conditions for 1 h at 40°C. The absorbance was measured at 464 nm. The activity of the dehydrogenase was expressed in μg of INTF g^{-1} h^{-1}.

The enzymatic activity of phosphatase was estimated according to the method of [45, 46]. The method is based on the spectrophotometric determination of p-nitrophenol released when the soil is incubated at 37°C for 1 h with p-nitrophenyl phosphate buffered solution; the alkaline solutions of this compound have a yellow color. Absorbance was measured at 405 nm. The activity of the phosphatase was expressed in μg of p-nitrophenol g^{-1} h^{-1}.

Urease was quantified by the technique mentioned in [47]. This technique is based on the determination of the ammonium released in the incubation of a soil solution at 37°C for 2 h. The determination of ammonium is carried out through the Berthrlot reaction with certain modifications. The Berthelot reaction consists of the reaction of ammonium in an alkaline medium with a coloring agent giving monochloramine, which is transformed into 2,2-isopropyl.5,5-methyl-indophenol when thymol is added. In this method, the ammonia produced by the urease activity reacts with salicylate and dichloro isocyanate giving a bluish green color. Absorbance was measured at 610 nm. The activity of urease was expressed in μg of NH_4-N g^{-1} 2 h^{-1}.

The respiration and the concentration of the enzymes in the soil were determined 7 days after their collection. The concentrations were adjusted according to the moisture content. All determinations were made in triplicate in a spectrophotometer (BioTek Synergy H1). In our experiments, all the reagents were analytical grade.

2.6. Statistical analysis

The median and the interquartile range are reported. To compare the concentrations of As, Pb, physicochemical parameters and biological activity (respiration and enzymatic activity) by the distance to the source, the Kruskal-Wallis test was used in logarithmically transformed data. Linear models based on distance (DistLM, models based on Euclidean distances with "step-wise" procedure obtained by 9999 permutations and selected under the criterion of best r^2) were performed to evaluate the association between biological activity and physicochemical parameters, as well as polluting elements. The DistLM model was calculated with logarithmically transformed and normalized data, excluding electrical conductivity due to its high association with the rest of the co-variables (As, Pb, pH and OM). In order to visualize the ordering patterns of the samples and the relationships between the variables, a redundancy analysis (dbRDA) was performed, representing the axes of greatest variation and the correlation between the covariates. The univariate analysis was performed using the GraphPad Prism Version 6 software, and the multivariate was performed using the software PRIMER 6 Version 6.1.18 & PERMANOVA Version 1.0.8 of PRIMER-E Ltd. Statistical significance was determined at 5%.

3. Results and discussion

3.1. Arsenic and lead in soil

The average content of As in the Earth's crust is 1.8 mg/kg, and, that of the soils has been found to be at 6.83 mg/kg. The most mobile forms of As are absorbed in a pH range of 7–9. The ions of As are known to be easily fixed in soils by Fe and Al hydroxides, the clay fraction, P and Ca compounds and organic matter [48]. The arsenic concentrations in this study ranged from 4.7 to 463.2 mg/kg. Changes in arsenic concentration were found mainly between the distances of 0–100 m and 1000–10,000 m ($\text{kW-H}_{4,25} = 21.71$ $p = 0.0002$, **Figure 3a**); with the highest concentrations being from the samples located in the first 100 m from the source.

Pb is a toxic metal naturally present in the Earth's crust (15 mg/kg). Mining, metallurgy, manufacturing and recycling activities, and, in some countries, the use of lead paints and gasolines, are among the main sources of environmental pollution [49]. The average level of Pb in soils has been measured at 27 mg/kg [48]. In our study, lead concentrations (mg/kg) ranged from 171.7 to 2319.0; the zones located between distances of 0 and 10,000 m showed the greatest difference ($\text{kW-H}_{4,25} = 13.61$ $p = 0.0086$, **Figure 3b**). Arsenic and lead concentrations of the sites located from 0 to 100 m from the source are above the levels established for the remediation of contaminated soils dictated by Mexican regulations (NOM-147-SEMARNAT/SSA1–2004), which establishes the limits at 22 mg/kg of As and 400 mg/kg of Pb.

In Villa de la Paz, Martínez-Toledo et al. [50] found values (mg/kg) of As from 1461.9 to 28274.0 and of Pb from 466.1 to 3486.4 in a site close to the source of contamination. In another study, Márquez-Reyes [51] determined the average total concentrations (mg/kg) of 13,443.4 of As and 1301.7 of Pb. González-Mille [52] reported average concentrations (mg/kg), for the site, of 222.1 of As and 204.3 of Pb. Chipres [53] found the presence of heavy metals of environmental

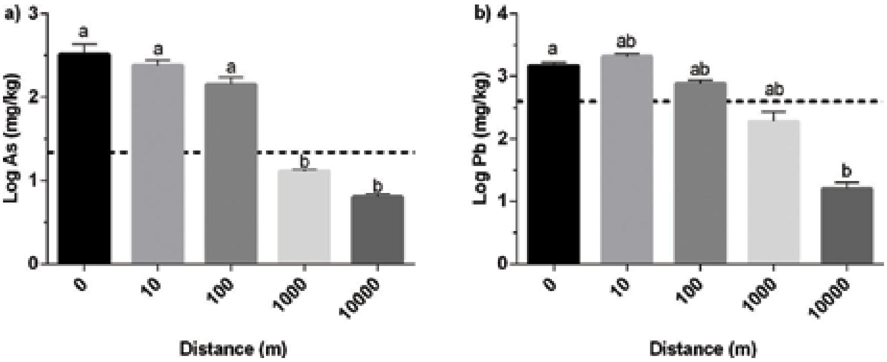


Figure 3. Comparison of As (a) and Pb (b) in distance gradient. The bars indicate the median, and the error bars the interquartile range. Different letters indicate the significant differences ($p < 0.01$) by the distance to the source. The dotted line represents the Mexican normative.

interest related to the geochemical state of the Altiplano showing ranges (mg/kg) from 4 to 324 for As. Due to the above, the values found in this study are similar and even lower than those found in previous studies for the area. In a study similar to ours, in this regard, Puga et al. [54] evaluated soil contamination by Pb, Cd, Zn and As at different distances (300, 600, 900, 1200 and 1500 m) and soil depth levels (0–40, 40–60, 60–80 cm), in an area of influence of mining waste in San Francisco del Oro, Chihuahua; the results showed that at a greater distance from the tailings, the concentrations of As and Pb decreased in a similar way to the one found in this study.

3.2. Physicochemical parameters

The pH levels in this study ranged from 3.9 to 8.2. A pH decrease was found in the distances of 0 and 10 with respect to the distances of 1000–10,000 m (kW-$H_{4.25}$ = 19.61, p = 0.0006, **Figure 4a**). According to Mexican regulations (NOM-021-SEMARNAT-2000), pH levels in soils in zones 0 and 10 m can be considered as strongly acidic and moderately acidic, respectively; while for distances from 1000 to 10,000 m, soil can be considered neutral. The pH values found were lower than those in other studies [50, 55, 56] for the area ranging from 7.1 to 8.2%, which may be due to the technique of pH determination or the selection of sampling points. pH is one of the most important parameters that control the change of the chemical forms of the elements in soil [57]. According to Rieuwerts et al. [58], the acid pH influences the absorption of heavy metals by plants and other organisms because the solubility of metals tends to increase at low pH values and decreases when pH is high. Near a neutral pH, the formation of complexes can become a metal immobilization mechanism, meaning that they have a low bioavailability [59]. According to what has been described above, it can be assumed that in the samples near the source of contamination (0 and 10 m), there is a high bioavailability of metals due to the low pH reported.

The EC varied from 82.8 to 1660 (μS/m). An increase of the EC was found mainly between the distances of 0–100 and the distances of 1000–10,000 m (kW-$H_{4,25}$ = 21.06, p = 0.0003, **Figure 4b**). EC levels can be considered as negligible effects of salinity for all sites in accordance with

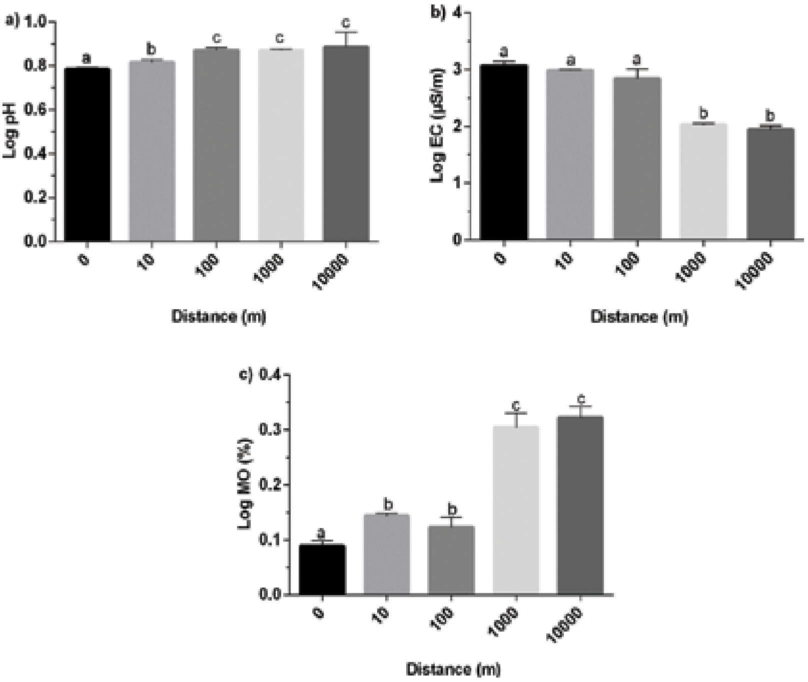


Figure 4. Comparison of elements and physicochemical parameters in distance gradient. a) pH, b) electric conductivity and c) 0rganic matter. The bars indicate the median and the error bars the interquartile range. Different letters indicate the significant differences ($p < 0.01$) by the distance to the source.

Mexican regulations (NOM-021-SEMARNAT-2000). The EC values found in this study were low with respect to other studies [50, 55, 56] done in the area ranging from 2200 to 12,200 μS/m; the differences could be due to the technique of determining EC or the selection of sampling points. A high EC could facilitate the mobility and bioavailability of metals in the soil through two possible means: (1) positively charged ions associated with salts (Na and K) that can replace heavy metals in the absorption sites and (2) negatively charged ions (for example chlorides) which can form stable soluble compounds with heavy metals (Cd, Zn and Hg), and have a tendency to generate acidic soils [59], as could be the case of the first 100 gradient meters.

OM varied from 0.8 to 1.2%. A significant reduction of OM was found mainly between the distances of 0 and 10–100 m and the distances of 1000–10,000 m ($kW\text{-}H_{4,25} = 21.49$, $p = 0.0003$, **Figure 4c**). OM is very low in the distances from 0 to 100 m and low in the distances of 1000–10,000 m in accordance with Mexican regulations (NOM-021-SEMARNAT-2000). OM values found were comparable with other studies [50, 55, 56] done in the area ranging from 1.2 to 11.6%. Organic matter is formed by humic acids that provide a broad cation exchange capacity [60]; the interaction between organic matter and clays can improve soil adsorption capacity of inorganic contaminants [61]. It has been shown through various studies that the addition of organic matter to the soil (compost, waste, leaf litter) can help to reduce the toxicity of heavy metals regarding the enzymatic activity of the soil, as well as their bioavailability [48, 62].

3.3. Respiration and enzyme activities

The activity of arylsulfatase varied from ND to 11.6 (μmol of p-nitrophenol g^{-1} h^{-1}). A significant decrease in the activity of the enzyme was found in the distances of 0–100 m and the distances of 1000–10,000 m (kW-$H_{4,25}$ = 21.86, p = 0.0002, **Figure 5a**), which represents an inhibition of 98.3%. The activity of Arylsulfatase was higher than that found by Montes-Rocha [55] for the area (ND at 0.68 μmol of p-nitrophenol g^{-1} h^{-1}); but the inhibition pattern in the enzyme was similar (98.8%). Borowik et al. [63] determined that arylsulfatase can be inhibited (85.7%) by high concentrations of Zn (2400 mg/kg). Hernández et al. [64] found an inhibition (64.3%) in the activity of arylsulfatase in a highly contaminated soil (mg/kg) by Zn (18900), Pb: 4930, Cd (15.10) and Cu (11.90).

The activity of β-Glucosidase ranged from 0.02 to 63.9 (μmol of p-nitrophenol g^{-1} h^{-1}). A significant decrease in the activity of the enzyme was found in the distances of 0–100 m with respect to the distances of 1000–10,000 m (kW-$H_{4,25}$ = 18.60, p = 0.0009, **Figure 5b**), which represents an inhibition of 99.4%. The activity of β-Glucosidase was higher than that found by Montes-Rocha [55] for the area (0.002–0.132 μmol of p-nitrophenol g^{-1} h^{-1}), and; the inhibition pattern in the enzyme was lower (86.4%). Experimental studies in soil contaminated mainly with Cu (450 mg/kg [65]), Zn (2400 mg/kg [63]) and mixtures of elements (Zn: 18,900, Pb: 4930, Cd: 15.10, Cu: 11.90 mg/kg), showed inhibitions of β-Glucosidase with respect to the control sample from 36.2 to 89.0%.

The activity of the dehydrogenase varied from ND to 0.44 (μmol of INTF g^{-1} h^{-1}). A significant decrease in the activity of the enzyme was found in the distances of 0–100 m with respect to the distances of 1000–10,000 m (kW-$H_{4,25}$ = 2.19, p = 0.0002, **Figure 5c**), which represented an inhibition of 97.4%. The activity of dehydrogenase was higher than that found by Montes-Rocha [55] for the area (0.001–0.003 μmol of p-nitrophenol g^{-1} h^{-1}), and the inhibition pattern in the enzyme was considerably lower (18.4%). Kucharski et al. [66] found an inhibition (34.3%) in dehydrogenase in laboratory studies due to exposure to Ni.

The activity of the phosphatase ranged from 0.04 to 0.82 (μmol of p-nitrophenol g^{-1} h^{-1}). A significant decrease in the activity of the enzyme was found in the distances of 0–100 m with respect to the distances of 1000–10,000 m (kW-$H_{4,25}$ = 18.46, p = 0.001, **Figure 5d**), which represented an inhibition of 79.6%. The activity of the phosphatase was similar with respect to that found by Montes-Rocha [55] for the area (0.017 to 0.103 μmol of p-nitrophenol g^{-1} h^{-1}), and the inhibition pattern in the enzyme was considerably lower (1.7%). Forty percent of inhibition of phosphatase is being found by exposure to Cd [67].

The activity of urease varied from 0.109 to 39.53 (μmol of NH_4-N g^{-1} 2 h^{-1}). The concentration gradient in the activity of the enzyme was found (kW-$H_{4,25}$ = 21.92, p = 0.0002, **Figure 5e**) in the following order: 0–100 < 100 < 1000 < 10,000 m. The inhibition pattern of the distances of 0–100 and 100 m with respect to 10,000 m was 99.9 and 96.7%, respectively. The activity of urease was lower than that found by Montes-Rocha [55] for the area (ND at 0.63 μmol of NH_4-N g^{-1} 2 h^{-1}), and the inhibition pattern in the enzyme was similar (100%). There are numerous studies of the inhibition of urease by different metals. In this regard, Belyaeva et al. [68] found urease inhibitions of 82.3 and 93.6% by Zn (8100 mg/kg). Yang et al. [69] showed that urea was inhibited by 75.1% with the presence of Cd (100 mg/kg) and Pb (500 mg/kg) in a controlled experiment. Kim et al. [70] found inhibitions from 21.5

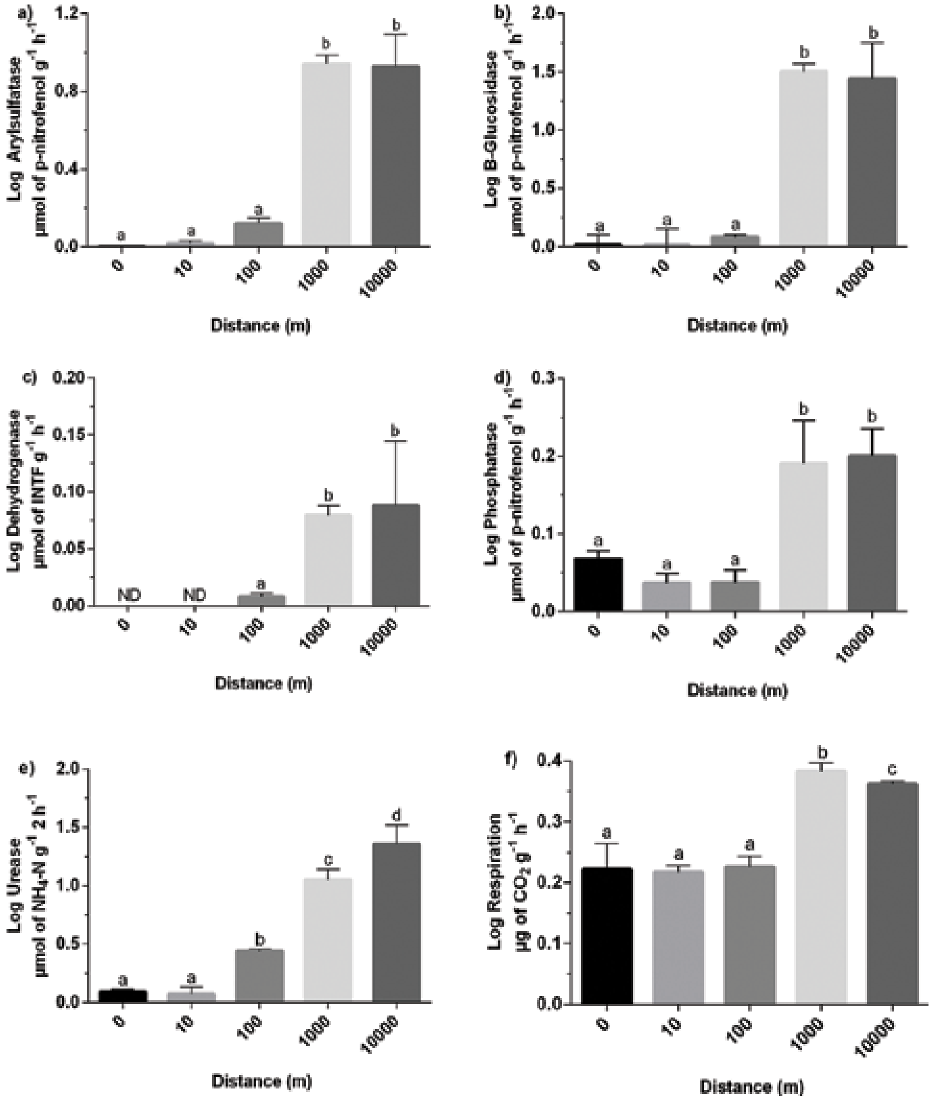


Figure 5. Comparison of enzymes and soil respiration in distance gradient. a) arylsulfatase, b) β-glucosidase, c) dehydrogenase, d) phosphatase, e) urease and f) respiration. The bars indicate the median, and the error bars the interquartile range. Different letters indicate the significant differences ($p < 0.01$) by the distance to the source. ND: Not detectable.

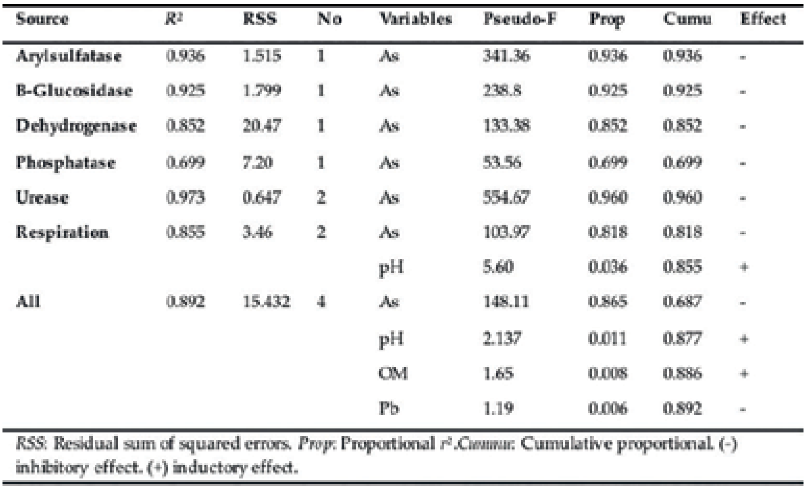
to 37.7% caused by Cu (200 mg/kg) in two types of soil. Researchers observed an inhibition of urease (65.3%) caused by Cu (800 mg/kg) in agricultural soils [71]. Borowik et al. [63] determined that urease can be inhibited (74.7%) by high concentrations of Zn (2400 mg/kg). Wyszkowska et al. [72] found that urea activity in agricultural soil can be inhibited (34.6–78.3%) by different mixtures and concentrations of heavy metals.

The production of CO_2 in soil varied from 0.62 to 1.5 (µg of CO_2 g^{-1} h^{-1}). A significant decrease in respiration was found mainly between the distances of 0–100 and the distances of 1000–10,000 m (kW-$H_{4,25}$ = 18.64, p = 0.0009, **Figure 5f**), which represents an inhibition of 47.7%. There are no respiration data in soil samples from the area. Speir et al. [73] found inhibitions in basal soil respiration due to addition of As.

3.4. Relationship between toxic elements and biological activities

The activity arylsulfatase (93.6%), β-glucosidase (92.5%), dehydrogenase (85.2%), phosphatase (69.9%), urease (96.0%) and respiration (81.8%) was mainly explained by the variations in As concentrations (**Table 1**). Few studies have been done on the effects of As in soil biological activity; in this regard, Speir et al. [73] found inhibitions in sulfatase, phosphatase, urease and basal respiration of soil due to addition of As (concentrations of 0–50 (μmol As [V] g^{-1} soil). Researchers conducted studies in soils of the USA contaminated by mixtures of toxic elements (among them 5.64 mg/kg of As and 250 mg/kg of Pb) in which they found a strong inhibition of β-glucosidase and phosphatase [4]. A slight negative effect of Pb (1.2%) is present in urease. Speir et al. [73] showed that urease was inhibited by 75.1% with the presence of Cd (100 mg/kg) and Pb (500 mg/kg) in agricultural soil in a controlled experiment. A 3.6% decrease in soil respiration can be associated with the change of pH of the soil, as mentioned above acidity increases mobility and bioavailability of the toxic elements and has a direct effect on microorganisms.

Regarding the total biological activity (dbRDA, **Figure 6**, **Table 1**), the grouping of the total samples into two large clusters corresponding to the distances 0–100 and 1000–10000 is shown as well as a greater dispersion in the information between the group of samples closest to the source of contamination. The direction of the vectors shows the influence of the variables toward the samples and the angles of the vectors represent the correlation between the variables. The DistLM model explained 89.2% of the total biological activity (**Table 1**).



Source	R^2	RSS	No	Variables	Pseudo-F	Prop	Cumu	Effect
Arylsulfatase	0.936	1.515	1	As	341.36	0.936	0.936	-
B-Glucosidase	0.925	1.799	1	As	238.8	0.925	0.925	-
Dehydrogenase	0.852	20.47	1	As	133.38	0.852	0.852	-
Phosphatase	0.699	7.20	1	As	53.56	0.699	0.699	-
Urease	0.973	0.647	2	As	554.67	0.960	0.960	-
Respiration	0.855	3.46	2	As	103.97	0.818	0.818	-
				pH	5.60	0.036	0.855	+
All	0.892	15.432	4	As	148.11	0.865	0.687	-
				pH	2.137	0.011	0.877	+
				OM	1.65	0.008	0.886	+
				Pb	1.19	0.006	0.892	-

RSS: Residual sum of squared errors. *Prop*: Proportional r^2.*Cumu*: Cumulative proportional. (-) inhibitory effect. (+) inductory effect.

Table 1. Results of the distance-based multivariate model (DistLM) for enzyme activity.

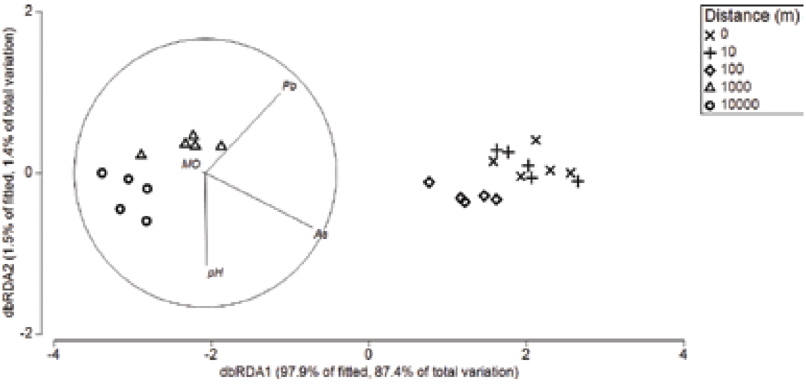


Figure 6. Distance-based redundancy analysis (dbRDA) ordination plot for biological activities (soil respiration and enzymes) with elements and covariables.

The predominant variables that negatively affect the total biological activity were electrical conductivity As, pH and Pb (88.4%) while OM (0.8%) has a positive effect.

Different studies show that enzymatic activities of the soil are inhibited by trace elements, for example, α and β-glucosidases, α and β-galactosidases, urease, phosphatases, arylsulfatase, nitrate reductase, l-glutaminase, l-asparaginase, L-aspartase and cellulase [74]. Toxic elements can modify the biological properties of the soil such as microbial activity, enzymatic activity and respiration [75]. Metals and metalloids are a fundamental part for the functioning of an ecosystem; however, when the concentration of these elements rises, they can cause great alterations at different scales. The biological activity of the soil can be seriously affected due to concentrations of certain heavy metals such as Cu, Ni, Cd, Zn, Cr, As and Pb. Several authors have shown that under certain concentrations and conditions, heavy metals and metalloids can cause harmful effects in the soil ecosystem [62]. These can inhibit enzymatic activity in an irreversible or reversible manner occupying the active place of the enzyme that the substrate would occupy preventing in this way the union with the substrate avoiding the formation of the products. Different authors have documented that the inhibition takes place at the level of the SH group. This mechanism occurs in each of the enzymes [76, 77].

Several authors have mentioned that the enzymatic inhibition mechanisms are as follows: (1) by interacting with the enzyme-substrate complex, (2) by denaturing the enzyme protein and (3) by interacting with active protein groups [3, 76]. In places like Villa de la Paz, these processes can be performed chronically for more than 200 years.

The inhibition of enzymatic activity cannot be attributed only to heavy metals since there are other variables that can influence this activity such as pH. Acosta-Martínez and Tabatabai [74] found that β-Glucosidase is sensitive to changes in soil pH and soil management practices, as well as Sherene [59] found that pH can affect the biological activity of the soil as well as the availability of nutrients. Another property that must be considered is organic matter because they can form complexes with heavy metals and enzymes and in this way reduce their toxicity. Martínez-Toledo et al. [50] found an increase in several enzymes directly associated with pH and OM content in mining sites of San Luis Potosí.

4. Conclusion

The amount of As and Pb detected in this study showed levels that were above the national criteria (NOM-147-SEMARNAT/SSA1–2004) mainly for the distances of 0–100 m, which reflects the danger of these elements for the environment. The historical mining activity in this site has left large amounts of mine tailings rich in heavy metals exposed to the elements, which are currently causing a problem of contamination and health impacts of soil, plants, animals and humans. The levels identified in this study justify immediate intervention in the area close to the tailings, followed by long-term monitoring for the protection of human and ecological receptors, as well as avoiding the mobility of contaminants to different environmental matrices (water and sediments).

The contamination by heavy metals and the effect of the mining activities in Villa de la Paz over time have caused for the physical–chemical properties (organic matter, electrical conductivity and pH), of the soils developed on the mining residues, to be significantly different to those of soils without the influence of pollution. These properties are involved in the interactions between microorganisms, enzymes and roots of plants, which are responsible for maintaining adequate soil health. It is recommended to carry out tests to evaluate the degree of degradation in at least 1 km around the mining waste to identify the alterations caused by the mining activity punctually and thus propose methods of soil recovery.

The biological activity of the soils presented a strong affectation due to the presence of heavy metals, which proved its usefulness as an indicator for rapid, reliable and low-cost evaluation. Levels such as those registered in the evaluated sites reduce the biological activity of soil in an alarming way, affecting their health, as well as the biogeochemical cycles and the environmental services that this provides. It was determined that metals are not the only ones who can negatively affect enzymatic activity, but also electrical conductivity and pH can interact in the phenomenon of inhibition. The organic matter with its capacity of immobilization of heavy metals promotes the increase of enzymatic activity.

The general pattern of inhibition for the area was urease>glucosidase>arylsulfatase>dehydrogenase>phosphatase>respiration; the latter being the least sensitive to inhibition. The continuous monitoring of parameters in this area is recommended. The alteration of these enzymes could be interrupting the biogeochemical cycles, as well as the material and energy flows of the ecosystems, making it more difficult to recover key processes for the maintenance of life such as the fixation of nitrogen and CO_2.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this chapter.

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Chapter 4

Flow in Unsaturated Soils and Transport of Herbicides in Agricultural Areas

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Additional information is available at the end of the chapter

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Abstract

Predicting the behavior of pesticides in the environment is necessary to minimize the adverse effects of their application. This means that we have to understand what is happening and how it is applied in the field and predict its fate in the environment to estimate the adverse effects on the subsoil and surface water or on human health. Knowing the level of contamination in soils involves characterizing the movement of water in the transport zone and solutes. The objective of this chapter is to disseminate and highlight the problem of the transport of pollutants in soils and to guide with regard to the available methods to obtain the necessary parameters to achieve their modeling and numerical analysis. To this end, the transport equation applied to the transport of agrochemicals is described and analyzed, and the methods of obtaining the parameters required by the mathematical solution are described. The general characteristics of the different herbicides and the environmental impact that the production of these compounds is producing are also described.

Keywords: unsaturated soils, transport of contaminants, agrochemicals, pollution

1. Introduction

Among the most important global problems affecting the environment are climate change, environmental degradation, loss of biodiversity, water pollution, and the growth of potential conflict due to competition for the use of natural resources. Groundwater does not escape this problem.

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The pollution has dramatically impacted the aquatic habitat in many of the world's most important water bodies. The contamination of the subsoil and the aquifers has the peculiarity of being "silent" and not presenting samples that show the same but until its consequences are observed in the biotic media.

Groundwater is usually more difficult to contaminate than surface water, but when this happens, reversing the situation is a very complex solution. The subsoil waters have a very slow rate of renovation. It is estimated that while the mean time of water stays in the rivers is of days, in an aquifer it is from years to hundreds of years, which makes it very difficult to decontaminate it.

The greatest concern about groundwater contamination has focused on the pollution associated with human activities such as the disposal of waste (liquid and solid waste in landfills of urban waste, waste from the oil industry, waste from the mining industry, radioactive waste, etc.) or not directly related to the emission of waste (agricultural activities, mining, construction and inadequate maintenance of buildings, etc.). On many occasions the situation is aggravated by the late knowledge that the aquifer is deteriorating [1].

Adequate protection of groundwater resources should have as a priority to prevent the entry of harmful elements into groundwater. It is a priority then to carry out geological, hydrological, and hydrogeological studies and potential sources of contamination.

Among the diffuse pollution activities, a very important one is the excessive use of fertilizers and herbicides. This type of pollution can cause situations of concern over time, because it is slow but continuous and in very large areas.

Herbicides are substances, usually of organic origin, which are used mainly for the control of weeds in agriculture. To minimize the environmental impact of the same, its application must be made considering the conditions of the plants, the soil, and the environment and the use of procedures for which they were designed and thus obtain an optimal dosage. However rigorous the conditions of use, there is evidence of the presence of traces of herbicides and other pesticides even in nonagricultural areas, in the atmosphere, and in surface and underground water [2].

The presence of pesticides, and especially herbicides, in groundwater and aquifers represents a frequent situation, especially in those regions that have had or have a high consumption of pesticides [3]. This would rule out the possibility of using water without a purification treatment, given that concentration levels of pesticides have been detected ranging from only traces to high levels of concentration.

When agrochemicals exceed the limits for which they were intended, they constitute both a loss to the agrosystem and a source of contamination for adjacent systems. The level of risk of contamination of soil and water results from the combination of the pollutant load and the natural vulnerability of the environment to the said contamination [4].

In this context, the prediction of the behavior of pesticides released into the environment is necessary to anticipate, and therefore minimize, adverse impacts outside the point of application [5]. This means that we must understand what happens to a pesticide that has been applied in the field and predict its destiny in the environment. Using this information, the likely adverse impacts on surface or groundwater and/or on human health can be estimated.

To this end, the solute transport equation applied to the transport of agrochemicals is described and analyzed in the following points, and some methods of obtaining the parameters required by the mathematical solution are described. It also describes in a general way the main characteristics of different types of herbicides and the environmental impact that the transport of these compounds is producing.

2. Flows in unsaturated soils

Pesticides, fertilizers, and solutes in general get dissolved or dragged with water from the soil through the pores, so the knowledge and measurement of properties such as hydraulic conductivity and effective porosity within the soil are important. These properties depend on the geometry, interconnection, and distribution of pore size within the soil. The presence of interconnected macropores is directly related to the natural aggregation of the soil and can constitute preferential flow paths within the soil [6], which is considered today the main mechanism for the relatively rapid appearance of contaminants in groundwater.

The term preferential flow refers to the fact that the water that infiltrates does not have enough time to get in balance with the water that remains in the soil matrix moving more slowly. These preferential flows can occur, for example, in structured soils, where macropores (cracks, tunnels caused by macroorganisms such as worms and insects, holes in the roots) dominate the hydrogeology of unsaturated to saturated soil, particularly in fine-textured soils, and operate as routes of high flow conductivity through the densest and least permeable matrix of the soil [7].

These preferential flows can also occur in unstructured sandy soils in the form of unstable wetting fronts, which is caused by heterogeneous profiles such as interfaces in the horizons or by water repellency [8].

A consequence of preferential flows is that a heterogeneous front of solute penetration does not occur in the soil; this contradicts the simple (convection-dispersion) equation, which predicts a homogeneous infiltration front.

2.1. Processes of transporting a solute in the soil

The transport process of a solute in the soil will present: volatilization (transport of the solute to the atmosphere); runoff to water surfaces; and leaching into groundwater.

These processes are affected by diffusion, convection, and dispersion. In addition, there are other processes that add to the previous ones such as retention (adsorption or sorption) and chemical transformation.

The processes of chemical transformation can be catalyzed by the constituents of the soil or photochemically induced. Most pesticides are transformed mainly by biochemical processes through soil microorganisms with changes in the molecule toward simpler forms that can be of equal, less, or greater toxicity than the original, which determines in what form and for how much the solutes will be present on the ground [9].

2.2. Transportation of contaminants

The one-dimensional solute transport equation in an unsaturated medium, which is similar to that of saturated medium, is written as shown in Eq. (1):

$$\frac{\partial C}{\partial t} = -v\frac{\partial C}{\partial z} + D_H\frac{\partial^2 C}{\partial z^2} - \frac{\rho}{n}\frac{\partial S_i}{\partial t} \tag{1}$$

where v is the linear velocity, D_H is the dispersion coefficient, C is the concentration of the pollutant, z is the distance along the direction of the flow, S_i is the concentration of the pollutant adsorbed, ρ is the volumetric density, n is the porosity, where the first, the second, and the third terms of the right hand side refer to advection, dispersion, and adsorption, respectively.

To model the concentration of a pollutant substance subjected to adsorption processes, the advective-diffusive transport equation is used in one dimension expressed by Eq. (2):

$$\frac{\partial C}{\partial t} = \frac{1}{R}\frac{\partial C}{\partial z}\left(-v + D_H\frac{\partial C}{\partial z}\right) \tag{2}$$

D_H: dispersion coefficient

C: concentration of the pollutant

R: delay factor

v: linear velocity

The variation of the solute is difficult to know, so in order to solve the equation, different isotherms can be considered. For the isotherms of variation, linear, second order, or exponential approximations can be considered.

If we assume that the concentration of the solute in the solid and liquid phases is related by a linear adsorption isotherm, the delay factor R takes the following expression Eq. (3):

$$R = \left(1 + \frac{\rho K_D}{\theta}\right) \tag{3}$$

ρ: volumetric density

θ: volumetric moisture content

K_D: distribution or adsorption coefficient that characterizes the linear isotherm.

In order to find the numerical solution of the transport equation, it is necessary to know the form of the concentration of the solute in the solid phase (porous matrix) and liquid phase (water) and how is the variation of the solute and the physical properties of the soils and their moisture content.

2.3. Measurement of solute concentration

The concentration of fertilizers and pesticides in different environmental matrices is estimated based on sampling based on statistical methods. In general, due to the great spatial variability of the concentrations of the different pesticides in the environment, the values obtained in a sample are only an approximation to reality. Therefore, the greater or lesser degree of certainty in obtaining the collected data and their interpretation depends to a large extent on an adequate sampling, on the collection of the sample and on the preservation of these [10].

In the study of the adsorption of a pesticide in a soil, two laboratory techniques are used: batch experiences and experiences in columns. Batch experiences are designed to study the equilibrium of adsorption in a continuously stirred soil suspension. This situation is presented as a physical model of particles of completely dispersed soil, where the entire surface of the particles is exposed and available for interaction with contaminants. Experiences in batches are not representative of the natural conditions that represent the conditions of a closed system and offer adsorption to the greatest possible surface area and, therefore, the maximum possibility of interaction [11].

3. Measurement of moisture in the soil

The infiltration process in the soil can be modeled by the Richards equation whose solution implies knowing the hydraulic functions of the soil. These functions depend on some parameters they need for their calibration of the hydraulic properties, determined by means of measurements. The measurements of the hydraulic properties of the soil present numerous complications due to two important factors: the nonlinearity of the conductivity/ suction function and the nonlinearity of the moisture/suction function [12].

Given the problem of the heterogeneity of the porous medium, the modeling of the water-solute-soil-plant system is more complicated. Therefore, obtaining the parameters that allow adjusting the model as close to reality is what takes most of time and money.

The adequate measurement of the water content in soil plays a critical role for the estimation of water and energy balances, as well as for understanding the biological and chemical processes in the entire soil-plant system [13].

The evaluation of soil moisture content at different suction conditions in the field requires considerable time and effort, as well as equipment. The effort, time, and equipment needed will depend on the range of interest required for the data.

The surface and depth to study must be carefully defined. The surface to study will depend on the variability existing in the place. In certain occasions the characteristics of the soil-water vary more with the depth that with the area.

The moisture in the soil depends mainly on the texture or the particle size distribution. On the other hand, the content of organic matter and the composition of the solution phase can play a determining role in soil moisture function or retention function. Organic matter has a direct effect on the retention function due to its hydrophilic nature and an indirect effect due to the modification of the soil structure that can be affected by the presence of organic matter [12].

Currently there are different equipment and measurement techniques for moisture content such as capacitance, reflectometry, geo-electric (called dielectrics), and neutrons. Descriptions of them are developed in the following points.

3.1. Neutron probe

A neutron probe is a sophisticated and accurate piece of equipment that measures the moisture content in soil. It requires calibration and operation by a licensed operator.

This technique is based on the theory that fast neutrons are thermalized when they collide with a body of similar mass, such as hydrogen nuclei. The energy of the neutrons is transmitted to the protons which causes the neutron "bounce or shock" to be much lower.

The application of this technique consists of three steps: (1) emission of fast neutrons from a radioactive source, (2) attenuation of the velocity of the neutrons after successive collisions with the atoms at the point of emission, and (3) accounting for neutrons with attenuated velocity by a detector near the source.

By means of electrical impulses the neutrons captured by the detector are translated into a digital reading.

To convert the reading of the neutron probe to volumetric moisture, a calibration model is necessary, where the volumetric moisture of the soil is the main factor. There are some factors that influence measurements such as hydrogen from organic matter, chlorine, iron, and boron present in the soil, capable of attenuating neutrons and absorbing thermally neutron nuclei [14].

3.2. Tensiometers

Tensiometers are widely used to measure the available water content of the soil when the matrix potential is high [15]. These devices are simple, they are not very expensive, and they are very practical in agricultural systems.

They consist of a porous ceramic capsule permeable to water and solutes, connected to a pressure gauge by means of a transparent plastic tube that is filled with water, in such a way that the column of water in its interior forms a continuous with the water of the solution of the floor in the surrounding space, through the porous capsule.

The values obtained reflect the soil tension, are negative values, and their operating range is 0–80 kPa, below this value the water column breaks, penetrating the air and invalidating the following measurements.

The tensiometers are insensitive to the osmotic potential of water in the soil and therefore do not provide an adequate measurement of the water potential in soils with significant salinity.

Tensiometers are often used in combination with the neutron sprayer, resistance blocks, or psychrometers to cover the full range of soil moisture. They require relatively frequent maintenance, which consists of adding water plus a solution for the control of algae [14].

3.3. Granular matrix sensors

They have been developed recently (they were patented in 1985 and manufactured commercially since 1989). It measures the electrical resistance between two electrodes inserted in a small cylinder composed of a porous material. Each device is covered by a membrane consisting of a stainless steel coupling, externally covered by a rubber that makes the sensor more durable than the plaster block. However, the recorder is calibrated to give the value in water tension, by means of an equation that takes into account the temperature of the soil estimated or measured near the sensor. The size of the pores in the matrix is greater than that of the pores in the gypsum blocks, allowing greater sensitivity in the more humid range of water content in the soil [14].

3.4. Dielectric sensors

The TDR and FDR probes measure the dielectric constant of the medium, which is an intrinsic property of the medium. The FDR system calculates the humidity of a soil by responding to changes in the dielectric constant of the medium using a frequency domain reflectometry technique known as capacitance, while the TDR uses time domain reflectometry [16].

3.4.1. Time domain reflectometry (TDR)

The TDR system consists of an oscilloscope connected to two or three metal rods that are inserted parallel to the ground. If a difference of power is applied to one end of the rods, the energy is transmitted along the ends to the end, where they are reflected to the oscilloscope. In it, the evolution of the potential over time is measured.

Some equipment consists of two main parts: the electronic unit and the waveguides. The electronic unit contains the oscilloscope and the central processor, which controls all measurement, display, and storage functions. The waveguides can be installed horizontally or vertically and remain permanently on the ground to make periodic measurements in the same location or be used in a portable way.

The TDR uses a series of conversion tables to convert the dielectric constant to a percentage of moisture in the soil. There are different conversion tables to be used with the different types of waveguides. It is not necessary to have a different table for the different soil types since the dielectric constant depends more on the amount of water than on the other soil components. The apparatus calculates the average value of the humidity over the total length of the waveguides. The apparatus allows manual measurements or continuous measurements by connecting the fixed sensors to a data logger.

3.4.2. Reflectometry in the frequency domain (FDR)

The FDR method is also known as a capacitance probe. The electrodes and the adjacent floor form a capacitor whose capacity is a function of the dielectric constant of the soil. This is related empirically to the volumetric content of water.

A capacitance sensor requires a probe calibration for each floor and horizon to obtain an optimal measurement of volumetric moisture. The volume of soil measured is not dependent on the type of soil or water content and approaches a cylinder 10 cm high with a diameter of about 25 cm, assuming there are no spaces with air [17].

All capacitance sensors installed in floors, even with similar characteristics, must be calibrated with the aim of improving their accuracy given the influence on the measurement of other factors independent of the moisture content such as pH variability or electrical conductivity inside of the porous matrix.

Capacitance sensors are the most economical and easy to install. In addition, it allows a continuous recording of the moisture values in the soil, enabling direct information and in real time. They are very useful for the planning of alert monitoring systems. They can be used as substitutes for neutron probes.

4. Herbicides

Herbicides are products intended for the control of weeds of a certain crop that are not desired due to their negative impact on production and yields. These are within the group of phytosanitary products, which of the World Health Organization (WHO) are all those substances or mixture of substances, designed to prevent the action, control, or directly destroy weeds, insects, fungi, mites, molluscs, bacteria, rodents, and other forms of animal or vegetable life that may be harmful to both public health and agriculture.

The growth and development of the food industry have had an effect on the current daily diet, diversifying the foods available in the diet. This progressive increase in production has been accompanied by surveillance and food laws in the countries, regulating and unifying processes and products [18].

In general, all the herbicides belonging to the same generic group act in the same way, for example: once the effect of atrazine on a weed is known, it is also known how simazine, ametryn, and prometryn act, since all these products belong to the same family of triazines [19].

4.1. Mechanisms of action of herbicides

A common feature that may have the herbicides is that they act on physiological processes of plants, being its toxicity very low on other species.

The most useful way of classifying herbicides is according to their mode of action [20]. The mode of action is the sequence of events that occur from the absorption of the herbicide to the death of the plant.

Herbicides with the same mode of action have the same absorption and transport behavior and produce similar symptoms in the treated plants [21].

In addition, the classification of herbicides according to their mode of action allows us to predict, in general terms, their weed control spectrum, application season, crop selectivity, and persistence in the soil [22].

4.1.1. Inhibitors of photosynthesis

The inhibitors of photosynthesis can be classified into mobile or systemic herbicides and non-mobile or contact herbicides. The inhibitors of photosynthesis include the chemical families of the triazines, triazinones, triazolinones, phenylureas, and uracils and the contact ones to the nitriles, benzothiadiazoles, and amides [23].

It is subdivided into four groups, the first three being those that act on the luminous phase of photosynthesis. Besides, they are not only used in agriculture but also to clean land, railroad tracks, industrial zones, and warehouses [24].

(a) Herbicides that inhibit the transfer of electrons by inhibiting photosynthesis.

Ureas, uracils, and triazines correspond to this group of herbicides. This type of herbicides are applied to the soil and absorbed by the roots, transported via xylem to the aerial part, reach the chloroplasts of the leaves, and there inhibit the light phase. Any plant can be affected by this type of herbicide.

(b) Herbicides that uncouple the electron transport chain.

They have the ability to capture the electrons preventing oxidation and forming free radicals “superoxides.” Superoxides are very powerful oxidants, which oxidize the unsaturated lipids of the chloroplast membranes, losing structure, and the chloroplast stops working. These molecules are formulated as bromides and chlorides; they are very soluble and very easily absorbed by the roots.

(c) Herbicides that prevent the formation of adenosine triphosphate (ATP).

Acylanilides, hydroxybenzonitriles, dinitrophenols, pyridazines, N-phenylcarbamates belong to this group. In photosynthesis, ATP is synthesized from the thylakoid membrane (sites of the photochemical reactions of photosynthesis) of chloroplast cells of plants. The photon particles from the sunlight excite the chloroplast thylakoid membrane, which in turn converts this excitation into ATP’s chemical energy. Found in all forms of life, ATP is often referred to as the “molecular unit of currency” of intracellular energy transfer. It is used by the plant for metabolic processes. They are applied differently. They can present an important toxicity for animals. Hence, some can be used as herbicides and fungicides.

(d) Herbicides that alter the biosynthesis of carotenoids.

They act at some point in the synthesis of lycopene. The most important is the amino triazole.

The most used inhibitors of photosynthesis are type (a).

4.1.2. Those that alter the biosynthesis of metabolites other than carbohydrates

They are subdivided into three groups:

a. Herbicides that alter the biosynthesis of aromatic amino acids: These amino acids are then part of proteins. The herbicide capable of inhibiting the synthesis of aromatic amino acids is glyphosate. Glyphosate in animals can be degraded; on the ground it can be inactivated, so that environmentally it has very good behavior.

b. Herbicides that alter the biosynthesis of glutamine.

c. Herbicides that inhibit lipid synthesis: Thiocarbamates that inhibit the conversion of short-chain fatty acids into long-chain AG belong to this group. As a result, they slow down the growth of the vegetable. These molecules can be used in soil treatments for seeds that are germinating; thiocarbamates are easily degraded by enzymes and are poorly absorbed in the soil.

4.1.3. Plant growth regulators

They alter the elongation and cell division. When they are incorporated into a plant, they give rise to an abnormal growth of the plant, and as a consequence it causes deformations, lack of functionality, and the death of the plant.

(a) Herbicides that alter cell elongation

Cell elongation occurs by the action of the auxins in the meristematic cells. The main effect of the auxins is the elongation of the cells, mainly due to the fact that the cell wall becomes more plastic. These at high concentration have herbicidal effects, cause excessive cell elongation with malformations in the apices, and the death of the vegetable. They are not used too much since in their synthesis dioxins are released. They are contact foliar herbicides that act at the point where they fall, they do not translocate. This allows many dicotyledonous weeds to be controlled.

(b) Herbicides that inhibit the synthesis of gibberellins

Gibberellins are phytohormones responsible for the growth of the plant, since they give rise to the internodes having a certain length. If the synthesis of gibberellins is inhibited, the internode distance is shortened, giving rise to the stunting of the plant and loss of functionality, in addition the petioles shorten, and the root system increases. All this causes the plant to lose functionality and die. Chlormequat (is a quaternary ammonium salt) inhibits the synthesis of gibberellins.

(c) Herbicides capable of inhibiting cell division

There are many types, but the most important ones are the N-phenyl carbamates and maleica hydrazida.

They are used in the soil and have little mobility and alter cell division as they prevent the correct organization of proteins that are part of the microtubules of achromatic use; this causes cells with giant nuclei without functionality. These herbicides affect meristematic cells, preventing them from thickening, there is no cell differentiation, and the plant stops growing and dies.

4.1.4. Herbicides with other mechanisms of action

(a) Herbicides that cause disruption of the cell membrane

These are the so-called herbicidal mineral oils. They are complex mixtures of long-chain CH that come from the fractional distillation of oil + dry fraction of coal. They are substances with very soluble lipids. They are used as total herbicides; they are also used as selective herbicides in some crops when the crops resist these oils and only the weeds will be eliminated. They were the first substances used as herbicides.

(b) Herbicides that act on pigments

These are the inhibitors of carotenoid biosynthesis (PDS). They interfere in the formation of chlorophyll, either by inhibiting the synthesis of typhoid, of carotenoids, or of the amino acid histidine.

They are applicable to soil or foliage. The characteristic symptom is foliar albinism after application. They are important herbicides when plants are exposed to full sun and depend on the light intensity; its effect begins to be close to 2 hours when the amount of carotenoids has decreased enough [24].

(c) Herbicides with hormonal activity

These are the disruptors of cell growth. Synthetic Auxins belong to this class with probable action towards indoleacetic acid. These herbicides interfere in the synthesis of nucleic acids, controlling the protein synthesis in different stages; affecting the regulation of DNA during the formation of RNA, an effect that can be achieved by the depression of a gene or activation of RNA polymerase; or simply affecting the RNA message to proteins. They are characterized by having a greater phytotoxicity toward dicotyledons and Cyperaceae than toward grasses; they act as growth regulators; transport occurs via symplast with the assimilated from the source of production to the organs in consumption or storage. They generally exhibit a short residual effect. The grasses are tolerant because they do not have cambium, besides the knots and internodes hinder the arrival of the herbicide to the site of action [24].

5. Conclusions

Pesticides, fertilizers, herbicides, and animal waste are sources of contamination of groundwater of agricultural origin. These sources of agricultural pollution are very varied and numerous: spillage of fertilizers and pesticides during handling, runoff by loading and washing spray pesticides or other application equipment, and the use of chemicals up the slope and a few 100 meters of wells or well-feeding waters. Contamination can also occur when chemicals are stored in uncovered areas, which are not protected from wind and rain, or stored in places where groundwater flows from chemical storage to wells.

The movement of water in the soil is the main mechanism for the transfer of pollutants to surface and groundwater [25]. The physics of water in the soil and the movement of solutes can be used to determine the behavior of these materials.

The movement of solutes through the unsaturated, vadose zone is particularly important in relation to environmental and agronomic contamination [26].

The limited availability of data obtained from field studies represents the main limitation to assess the degree of contamination of groundwater against the effects of herbicide applications, especially in agricultural areas.

On the other hand, the scarcity of measurements makes it difficult to validate the mathematical models for determining the content of herbicides and fertilizers in groundwater and in the vadose zone. However, isolated data from groundwater in agricultural areas under the application of herbicides and fertilizers in recent years show that the waters of unconfined aquifers are contaminated by herbicides. In the medium and long term, this pollution will affect the environment in agricultural areas.

To minimize the environmental damage caused by the application of herbicides, its application must be carried out taking into account, strictly, the conditions of the plants, the soil and the environment, as well as the doses and proper procedures for use, but this is not enough.

The current challenge is to advance in the knowledge of solute transport processes and determine the contamination that can be submitted if continued or continued with the current application methodologies and their concentrations.

But what is more important is that we must not forget that the purification possibilities of an aquifer are limited and that the best method of protection is, therefore, prevention. Do not contaminate; control sources of contamination to know the effects well and avoid contaminants.

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Section 2

Alternatives for Sustainable Development

Chapter 5

Mitigation of Nitrous Oxide Emissions during Nitrification and Denitrification Processes in Agricultural Soils Using Enhanced Efficiency Fertilizers

Yoshitaka Uchida and Isabell von Rein

Additional information is available at the end of the chapter

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Abstract

Through the increasing use of nitrogen (N) fertilizers due to an increasing food demand, the agricultural sector is the main contributor of anthropogenic nitrous oxide (N_2O) emissions, mainly through microbial processes called nitrification and denitrification. One option to mitigate N_2O, a major greenhouse gas, is to use enhanced efficiency fertilizers (EEFs). There are different types of EEFs like nitrification inhibitors or controlled-release fertilizers that aim to match the N release from fertilizers with N demands from plants. Parts of the chapter are also dedicated to organic amendments and their effects on N_2O emissions. Overall, EEFs can improve the N-use efficiency of plants, which has two positive effects. First, farmers can increase their yields, and second, environmental pollution through excessive fertilizer N can be minimized. However, the effectiveness of EEFs strongly depends on numerous factors like land use type, application method, and climate. More studies are needed to establish individual fertilizer plans that are optimized for the prevalent conditions. In conclusion, N_2O mitigation using EEFs is only advisable when "initial" N_2O emissions from conventional fertilizers are critically contributing to annual N_2O emissions. Thus, careful assessment is needed before EEFs are introduced to the system especially when economic and ecologic results are considered.

Keywords: N_2O emissions, enhanced efficiency fertilizers, coated urea, nitrification inhibitors, organic amendments

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1. Introduction

1.1. Importance of fertilizer application

With an ever-increasing world population, modern agriculture faces the challenge to satisfy the increase in food demands. Fertilizers are indispensable for higher yields in agricultural food production [1]. They supply plants with primary nutrients like nitrogen (N), phosphorus (P), and potassium (K) with the focus of this chapter lying on N. Even though around 80% of the atmosphere consists of molecular N, it cannot directly be used by most plants due to it being highly stable and chemically inert [2]. Only a limited number of plants (legume plants) can fix atmospheric N through a symbiosis with N-fixing rhizobia bacteria called biological N fixation. The plant-microbe interaction results in new plant organs called root nodules, where the bacteria convert atmospheric N into available N for the plant [2, 3]. Thus, a regular replenishment of nutrients, e.g., in the form of manure, soil amendments, or organic or synthetic fertilizers, is necessary to keep the harvest quantity and quality of nonleguminous crops high [4]. The establishment of the Haber-Bosch process and with it the production of chemical N fertilizers markedly increased agricultural yields [1]. However, agricultural intensification comes with a price. To satisfy the large increases in food production, N production through the Haber-Bosch process increased by 20% from 100 in 1995 to 121 Tg N year^{-1} in 2005 and will continue to rise with estimates of about 145 Tg N year^{-1} in 2050 with N fertilizer production exceeding N fertilizer consumption by about 7% annually [5, 6]. This poses a huge threat to environmental systems because not all of the fertilizer N produced will actually reach the plants, be it through an overproduction of N or through direct losses of N to the environment after N fertilizer application.

1.2. Problems of fertilizer application

Only around half of the conventional N fertilizers applied are utilized by the plants, while the rest is lost to the environment, with around 3, 20, and 25% of N lost through accumulation in the soil, leaching to aquatic systems, and emissions to the atmosphere, respectively [6, 7]. Plants assimilate N from the soil solution as nitrate (NO_3^-) or ammonium (NH_4^+) ions. However, NH_4^+ can be adsorbed to the soil and is used in nitrification processes leading to a competition for N with the soil biome, whereas NO_3^- is negatively charged and easily lost from the soil (also negatively charged) through leaching [8]. The application rates of conventional fertilizers often exceed plant demands, and about 40–70% N is lost to the environment (**Figure 1**) [5, 6, 8]. Especially, N fertilizers are susceptible to loss because of processes such as leaching, mineralization, volatilization, and other gaseous emissions (e.g., ammonium (NH_3) and nitrous oxide (N_2O) emissions). As a consequence, N fertilizer application can lead to heavy environmental pollution due to excessive N not taken up by the plants. Two major processes of N pollution include NO_3^- leaching to the groundwater leading to eutrophication and N_2O emission to the atmosphere via nitrification and denitrification processes of soil microbes [4].

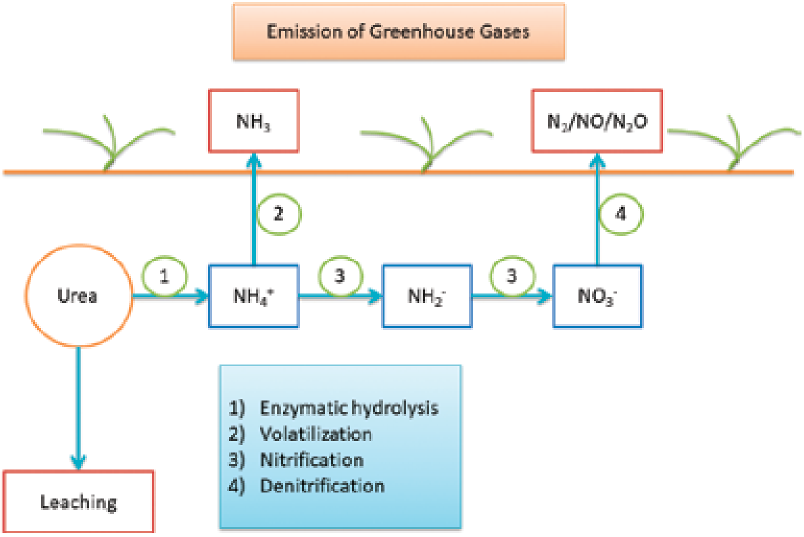

Figure 1. Losses of nitrogen (red boxes) after urea application through different processes. For simplification reasons some processes of N turnover have been omitted (e.g., steps in the denitrification process, aerobic, and chemo-denitrification).

1.3. Enhanced efficiency fertilizers (EEFs): possible solutions for fertilizer application

Countermeasures against the environmental impact of N from fertilizer N include the use of enhanced efficiency fertilizers (EEFs). There are several types of enhanced efficiency fertilizers (EEFs). Some contain nitrification or urease inhibitors (stabilized fertilizers), while others slowly release N components (slow-release fertilizers) or release N at more predictable rates (controlled-release fertilizers) [8–11].

The use of EEFs is primarily to improve the N-use efficiency of crops. Choosing the right type of EEFs can synchronize the demands of N by the crops and the release of N by the fertilizers [12]. As an alternative approach, farmers may apply normal and/or enhanced efficiency fertilizers several times during the crop-growing season based on the requirements of the crop which is called split application [13]. However, the split application is often labor-demanding and not cost-efficient. We note that farmers normally decide to use the EEFs based on their cost performance unless they are required to use EEFs due to some environmental regulations. Thus, the mitigation of N-related environmental impacts is a secondary benefit for the farmers. There are different types of EEFs available on the market. In the next part of this chapter, we list their characteristics.

1.3.1. Stabilized fertilizers

Fertilizers containing N stabilizers extend the time that the N added to the soil stays in its original form. Stabilized fertilizers like nitrification and urease inhibitors directly slow down microbial processes. For example, nitrification inhibitors (NIs) contain substances that inhibit ammonia monooxygenase—the first enzyme involved in the oxidation process of nitrification—and thus delay the NH_4^+ oxidation to NH_2^- by nitrifiers [8, 14, 15]. By slowing down the nitrification rate, plants have a higher chance of assimilating NO_3^- over a longer period which increases the N-use efficiency of the fertilizer. With reduced NO_3^- concentrations in the soil, NO_3^- leaching and N_2O loss via denitrification processes (which require NO_3^- as a substrate) are minimized [8]. In summary, NIs slow down the two main processes of N_2O production in soils with the indirect inhibition of denitrification being the most important effect since denitrification is the main contributor to N_2O emissions from agricultural soils [15]. NIs can be applied with chemical as well as organic fertilizers. Three commonly used NIs are N-(n-butyl) thiophosphoric triamide (NBPT), dicyandiamide (DCD), and 3,4-dimethylpyrazole phosphate (DMPP). NBPT is the most widely used NI due to it having a similar solubility and diffusivity as urea and thus a high effectiveness. However, under high temperature and humidity, e.g., under tropical conditions, its effectiveness might be reduced since it is rapidly degraded [8, 16]. Urease inhibitors suppress the hydrolytic action of the enzyme urease and therefore slow down the rate at which urea is hydrolyzed to NH_3 which reduces volatilization losses of NH_3 to the air [9]. In this chapter, the focus will lie on NIs since they are more promising in mitigating N_2O emissions than UIs [14].

1.3.2. Controlled-release fertilizers (CRFs)

Slow- and controlled-release fertilizer notations can be used interchangeably in most cases. One possible difference is that the nutrient release pattern of slow-release fertilizers is unpredictable due to the dependence on soil and climatic conditions, while it can roughly be predicted for controlled-release fertilizers [8]. Controlled-release fertilizers (CRFs) retard or control the release rate of nutrients to the soil which can lead to higher yields and a reduction of environmental pollution from nutrient loss through chemical modifications or coating. Coatings can be based on, e.g., sulfur, polymers, or superabsorbent/water retention materials with new and more accurate and efficient CRFs being developed on a constant basis [4, 8, 10, 11, 17, 18]. Coatings can only be applied to chemical fertilizers. Coated N fertilizers release N through a physical process like diffusion through the semipermeable coating, while slow-release fertilizers depend on chemical or biochemical processes [9]. One possible release mechanism of coated fertilizers is called multistage diffusion model where water penetrates the coating and condenses on the solid fertilizer core which dissolves the nutrients slowly. The osmotic pressure rises within the core, and the granules start to swell which leads to a slow diffusional release of the N due to a concentration and/or pressure gradient [10]. Since urea is one of the most widely used N fertilizers in the world, coated urea has been one of the most researched CRFs. Coated urea is a granule CRF which releases more N with increasing temperature and is designed to release N as close to the plant's N demand as possible. The N release can be controlled by altering the coating properties (e.g., the thickness) and thus changing the N diffusion rates. Coated urea reduces N_2O emissions by limiting the available N for microbial processes like nitrification and denitrification in the soil [9, 10, 17].

Advantages of EEFs over conventional fertilizers include higher N-use efficiency (nutrients taken up by plants compared with the amount applied) and N availability over the whole crop season. The resulting environmental benefits are decreases in NO_3^--N loss through leaching, NH_3 loss through volatilization, and N_2O emissions through microbial processes.

On the other hand, they are quite expensive, and their role in mitigating N_2O emissions is not yet fully understood with contrasting results [4, 9, 10, 17]. In conclusion, maximizing the N-use efficiency should be the goal when applying N fertilizers and EEFs in order to reduce environmental impacts like N_2O emissions and at the same time increase the yield.

1.3.3. Organic amendments

Even though organic fertilizers—also called organic amendments (OAs)—like plant residues, composts, or animal manure are not EEFs, they are often said to be more environmental-friendly than conventional fertilizers. The advantages of using OAs include improvement of soil C and N content, yield, and microbial biomass and activity. In addition, using OAs can recycle already fixed N [19]. Thus, animal manure and plant residue applications have been the traditional way of fertilizing agricultural fields for centuries, but recently—with the increasing demand of food—chemical fertilizers became more and more common. Still, due to the many negative effects for the environment of chemical fertilizers as well as their relatively high cost compared to OAs and unavailability in many local areas of the world, the use of OAs is rapidly increasing as well [20]. Negative environmental impacts of OAs include nutrient leaching and greenhouse gas (GHG) emissions, but their impact is much less severe than that of chemical fertilizers.

Animal manures, e.g., from livestock (cattle, pigs, or sheep) and poultry, are one of the most commonly used OAs that are rapidly increasing in quantity due to the increase in worldwide meat production. Animal manure has a substantial amount of nutrients like N, P, and K and can help to improve soil fertility and health [20]. Composts (e.g., made from crop residues or manure) are a good C source and help to enhance soil aggregate stability and prevent erosion, but most composts contain little amounts of nutrients, e.g., only 1–2% N (urea contains 46% N) which leads to the necessity of either additional addition of other fertilizers or high application rates. Also, during composting, high C and N losses occur through GHG emissions [19, 20]. Plant residues include stalks, stems, leaves, and seed pots and can function as a soil N source. Most plant residues are from cereals or legumes (e.g., red clover, winter vetch, and ryegrass). Their application method—e.g., surface application or incorporation into soil—as well as soil moisture and residue quality can have different impacts on GHG emissions with potential increases in denitrification after incorporation. Residues with a high N content and low C/N ratios like legumes might release N faster which can result in higher N_2O emissions, while high C/N ratios might decrease N_2O emissions through released N during residue degradation which stimulates microbial immobilization [20–23].

1.4. N_2O emissions

1.4.1. N_2O as a threat for climate and ozone layer

Nitrous oxide (N_2O) is a major GHG with a global warming potential around 298 times greater than that of CO_2 over a 100 year period [24, 25]. Due to the high global warming potential—which depends on its high potential of absorbing infrared light and a long atmospheric lifetime of 114 years—even low concentrations of atmospheric N_2O have strong negative impacts regarding climate change. Global emissions of N_2O occur mostly through biological processes.

Thus, factors affecting N_2O emissions from soils include oxygen (O_2) concentration, temperature, moisture content, soil texture and type, and soil-NH_4^+ and soil-NO_3^- concentrations that are readily available for nitrification and denitrification [26, 27]. In addition, soil organic C content and soil drainage as well as microbial community structure, abundance, and activity influence the magnitude of N_2O emissions. Agricultural management-related factors controlling N_2O emissions include fertilizer and crop type, tillage system, as well as N application rate, timing, and technique [26, 27]. It is estimated that—due to human activities—N_2O emissions have increased by more than 40% compared to preindustrial levels [27]. For example, [25] estimated that global emissions have increased from 12 in 1500 to 19 Tg N year^{-1} in 2006, with natural emissions accounting for about 55% and anthropogenic emissions accounting for about 45% in 2006.

Next to its global warming potential, N_2O also has a huge effect on the ozone layer. It is said to be the main ozone-depleting substance today and will remain the largest threat to the ozone layer throughout the twenty-first century if the current emission trend continues. Nitrous oxides (NO_x) catalytically destroy the ozone layer, and their main source is surface N_2O emissions. N_2O already outweighs the chlorofluorocarbons—the historically dominant ozone-depleting substances—because of its abundance and continued anthropogenic emissions as well as a high ozone depletion factor of 0.017 [28]. Thus, N_2O emissions affect both the ozone layer and our climate. We need to be aware that when fighting climate change through the mitigation of CO_2 emissions, N_2O emission could unintendedly rise, e.g., as a consequence of enhanced crop growth for biofuel production [28]. To avoid exchanging one GHG with another, we need to mitigate anthropogenic N_2O emissions which mainly arise from N fertilization.

1.4.2. N fertilization as a source for microbially produced N_2O

The agricultural sector (including livestock production systems) is responsible for about 42% of the global anthropogenic N_2O emissions [15, 25, 29]. Agricultural soils are the main contributor to anthropogenic N_2O emissions, and it is estimated that emissions will continue to rise in the future (**Figure 2**) mainly due to increases in N fertilizer use and manure application [30]. The most commonly used N fertilizer is urea ($CO(NH_2)_2$). When applied to the soil in the presence of water, an enzyme called urease converts urea into NH_4^+, hydroxyl ions (OH^-), and bicarbonate (HCO_3^-) [10, 17, 27]. The N_2O emissions from N fertilization (primarily the addition of chemical fertilizers and organic fertilizers like animal manure) mainly arise from microbial processes called nitrification (aerobic) and denitrification (anaerobic). Nitrification is the aerobic process of catabolic oxidation where NH_4^+ is firstly transformed to nitrite (NO_2^-) by *Nitrosomonas* sp. and then to NO_3^- by *Nitrobacter* and *Nitrosolobus* sp. bacteria. It is mainly influenced by the availability of NH_4^+ and O_2 [26, 27]. Also, many archaea species oxidize NH_4^+, and a recent study discovered that there are *Nitrospira* species that can perform the complete nitrification process (the two steps mentioned above) on their own [31]. Nitrification is a very important microbial process in the soil—especially for plant productivity and environmental quality—as it determines the form of N present. Nitrification converts the relatively immobile NH_4^+ to the highly mobile NO_3^-. NO_3^- is often the major compound utilized by plants due to it being easily available, but at the same time, it can also be easily lost through leaching and denitrification [8, 27, 32].

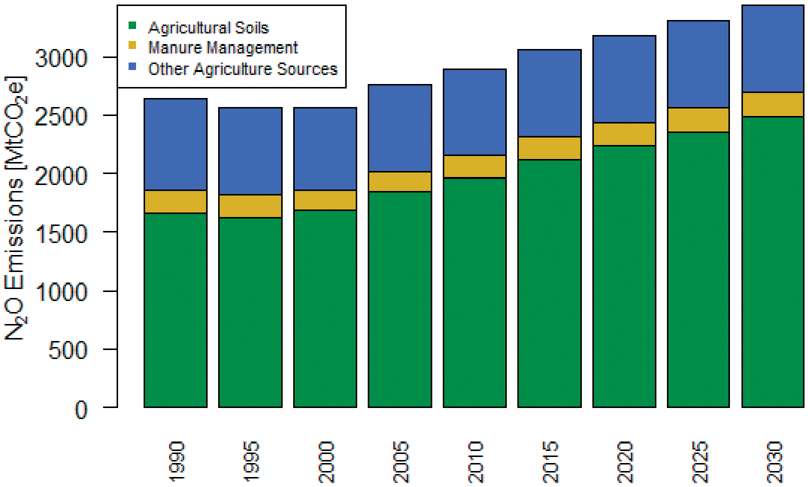

Figure 2. Global N_2O emissions from agriculture between 1990 and 2030 (data obtained from [35]).

Denitrification is the anaerobic process of the reduction of NO_3^- to N_2 via NO_2^-, NO, and N_2O with high losses of N_2O to the atmosphere. Denitrification is much more temporally and spatially variable than nitrification and is influenced by NO_3^-, C, and O_2 availability [8, 26, 27]. A very important, yet often overlooked, process of N_2O production is called nitrifier denitrification which, under some circumstances, can even outweigh denitrification. It is the reduction of NO_2^- by NH_3-oxidizing bacteria. N_2O emissions from NH_4^+ might in some cases entirely emanate from nitrifier denitrification. Favorable environments for this process are low in oxygen and high in pH and have fluctuating aerobic-anaerobic conditions [33].

It is estimated that around half of the global anthropogenic N_2O emissions arise from N fertilization and it is thus a major concern in terms of atmospheric GHG increases [34]. The N_2O emission factor (EF) which is the "percentage of fertilizer N applied that is transformed into fertilizer-induced emissions" is estimated as 1% which means that from 100 kg of applied N, 1 kg is lost to the environment as N_2O emissions [34]. However, a 1% EF assumes a linear relationship between N application and N_2O emissions while [34] rather suggest an exponential curve which would indicate that global N_2O emissions are underestimated in general. In addition, it is predicted that agricultural N_2O emissions will increase by 20% until 2030 [35].

In order to mitigate N_2O emissions, we first need to quantify the N_2O budget and understand the mechanisms and processes linked with N_2O production. Stable isotope techniques offer a unique opportunity to gain insights into processes contributing to N_2O emissions, and recently laser spectroscopy approaches have highly increased our knowledge on the functioning and the controls regarding the N_2O budget [36].

By using isotopic ratio mass spectrometry, it is possible to estimate (e.g., through natural abundance measurements) and quantify (e.g., through enrichment experiments) the (microbial) processes associated with N_2O production based on ^{15}N and ^{18}O isotopes [37]. It is difficult to assign N_2O production to different processes mainly due to many simultaneous processes that contribute to N_2O production in different microsites of the soil. To attribute N_2O

emissions to denitrification, acetylene (C_2H_2) is added which inhibits nitrification. However, this method can underestimate denitrification because no additional NO_3^- is supplied via nitrification. In addition C_2H_2 can be used as a substrate if C in the soil is limited which can lead to biased results [37]. Natural abundance approaches can be used as an estimate of the N_2O sources in soil, but the results should be handled with caution due to a fractionation in favor of ^{14}N relative to ^{15}N and higher fractionation during nitrification than denitrification. Thus, even though natural abundance approaches have the advantage of being noninvasive, they are not suitable for exact N_2O source partitioning and thus best be coupled with genetic and/or molecular approaches [37].

2. Enhanced efficiency of N fertilizers and their role in mitigating N_2O emissions: comparing conventional, organic, and enhanced efficiency fertilizers

One option to reduce N loss to the environment as N_2O gas is to use EEFs. As previously described, EEFs match the N release from fertilizers and N demands by crops. Thus, the excess accumulation of fertilizer-derived N in soils can be avoided. This fact is very important to minimize the N_2O emissions from soils because N_2O emissions are related to the microbial availability of N in soils. Even though environmental issues are important, the main reason for applying EEFs in recent years was to increase the N-use efficiency through optimized N management practices, which, e.g., can reduce the number of split applications a farmer has to do [13, 34]. The main goal for farmers is thus to decrease the amount of work and at the same time increase the yield due to an optimized N fertilizer utilization rate. The main goal for humanity however should be to combine high agricultural yields with a minimum environmental pollution [15].

2.1. Stabilized fertilizers

In a meta-analysis by [14]—who evaluated the overall effectiveness of EEFs by comparing them with conventional fertilizers using datasets from field experiment data—they concluded that nitrification inhibitors (NIs) have the highest potential in mitigating N_2O emissions with a mean reduction of 38% (when compared to conventional fertilizers) and a relative constant effectiveness for different soil and land use types. The effectiveness of NIs to reduce N_2O emissions varied with land use type, e.g., mitigation in grasslands (−54%) was much higher than paddy fields (−30%). This indicates that the mitigation effectiveness of NIs depends on the mean N_2O emissions of the land use. The higher the mean N_2O emissions, the more effective are NIs in reducing them. However, in their meta-analysis, urease inhibitors (UIs) seemed to be ineffective in reducing N_2O emissions. In another meta-analysis by [32]—who assessed how NIs affect both hydrologic and gaseous N losses and N-use efficiency—NI application increased NH_3 emissions by a mean of 20% but reduced N leaching, N_2O emissions, and NO emissions by 48, 44, and 24%, respectively, which led to a total net reduction of 16.5% of N released to the environment. Thus, overall, NIs seem to be a good option in reducing N_2O emissions, but their effectiveness may vary strongly on a local scale.

Many studies have assessed the effectiveness of stabilized fertilizers, but the results vary. The application of DCD and/or DMPP has shown positive results in mitigating N_2O emissions in various studies [38–40]. For example, [38] found that DCD and DMPP, two NI fertilizers in combination with urea, decreased cumulative emissions by 35 and 38%, respectively, in a 1 year experiment on a wheat-maize rotation field when compared to only urea application. In addition, yield, aboveground biomass, and nitrogen uptake increased significantly when using NIs, possibly through an increase in soil inorganic nitrogen and a shift from NO_3^- to NH_4^+ forms. Other studies that assessed the effectiveness of DCD and/or DMPP concluded that they have a great potential to reduce N_2O emissions as well as NO_3^- leaching, e.g., from cow urine in grazed pastures or in intensive vegetable production systems [39, 40].

However, e.g., [41] found no significant reduction of N_2O emissions after urea + DCD application, even though urea + nitrapyrin did mitigate them in a 2 year study on an intensively used vegetable field. Recently, it is suggested that the positive effects of NIs on reducing N_2O emission might be overestimated or even reversed when the indirect N_2O emissions from increased NH_3 volatilization after NI application are considered [42]. A study by [16], who quantified N_2O emissions in a 1 month field trial with maize plants on a tropical acrisol after urea + NI fertilizer (nitrapyrin and NBPT) application, assessed this problem. They found that the urea + nitrapyrin treatment reduced cumulative N_2O emissions by 49%, while NBPT reduced the NH_3 volatilization by 35% compared to the urea treatment. NBPT tended to increase N_2O emissions; however, it reduced N_2O emissions indirectly by reducing NH_3 volatilization. In addition, while nitrapyrin was added after urea application during maize sowing, NBPT was added during the V5 stage of maize growth which increased maize yield significantly. The authors concluded that the combination of timing, placement, as well as the specific use of fertilizers are vital for the effectiveness of NIs to reduce N_2O emissions directly and indirectly through a reduction of NH_3 volatilization as well as for higher N-use efficiency and yields. Recent studies also suggest that—even though NIs like DCD and DMPP are a good option to reduce N_2O emissions—they might have little economic benefits since they are relatively expensive and might not increase biomass and yield as expected. This is due to the usage of suboptimal N rates in many studies that focus on the effects of N_2O emissions after NI application [43].

Thus, the effectiveness of stabilized fertilizers depends on many factors. For example, in systems with a high N surplus, N_2O mitigation can simply be achieved by optimizing the N input because N_2O emissions increase strongly if N application rates are higher than the N uptake. But EEFs can be an effective way to mitigate N_2O emissions in agricultural systems that are moderately fertilized and that are adapted to the N demand of plants [15]. Ref. [15] also suggest that the optimal N_2O reduction occurs under conditions where the NI remains close to the N fertilizer, e.g., when the NI is sprayed on mineral-N fertilizer granules or is thoroughly mixed with liquid fertilizers. Thus, on pasture soils with high spatial N concentration variations due to urine and manure patches, N_2O mitigation strongly varies depending on N_2O hotspots dispatched across the soil.

In general, when long-term experiments and meta-analyses are conducted, NIs usually show a positive effect on the mitigation of N_2O emissions. Thus, high-frequency measurements over a long time period are important to accurately quantify N_2O emissions and assess the overall effectiveness of NIs because with short-term experiments and low-frequency measurements effects may be over- or underestimated. This is due to the regulation of N_2O emissions via soil temperature, moisture, and inorganic N contents which leads to strong seasonal and even daily fluctuations [38].

In conclusion, N_2O mitigation using NIs is only advisable when "initial" N_2O emissions from conventional chemical fertilizers are critically contributing to the annual N_2O emissions. Thus, careful assessment is needed before NIs are introduced to the system especially when economic and ecologic results are considered.

2.2. Controlled-release fertilizers (CRFs)

Numerous studies have shown the effectiveness of CRFs like coated urea to mitigate N_2O emissions compared to conventional fertilizers [9, 44]. Ref. [9], for example, recorded a reduction of N_2O emissions of 42% with polymer-coated urea when compared with urea in no-till and strip-till environments for irrigated corn in Colorado. It was shown in a global meta-analysis that—while N_2O emissions from urea were high—coated urea had one of the lowest emission factors and manure was somewhat in the middle among all investigated fertilizers [34]. Ref. [14] found a reduction of N_2O emissions by 35% compared with conventional fertilizers when CRFs were applied. However, the effectiveness varied depending on the soil and land use type. CRFs were very effective for imperfectly drained Gleysol grassland (77% N_2O reduction) but ineffective for well-drained Andosol upland field. Land use type seems to be a strong factor controlling N_2O emissions after CRF application [14, 44–46]. Ref. [46] evaluated the effects of soil and fertilizer types on oil palm plantations using urea and coated urea and sandy loam, sandy, and peat soil. They found high variations in the N_2O emissions dependent on the treatments with sandy soil emitting the lowest and peat soil the highest emissions overall. Coated urea reduced N_2O emissions by around 40% in the sandy loam soil compared to the conventional fertilizer, but emissions increased in the other soils during dry season. In a study that tested an NI (DCD) and coated urea on two contrasting soils, Andosol and Fluvisol, the NI only significantly reduced N_2O emissions (compared to urea) in Andosol, and nitrification was the dominant process compared to Fluvisol. For coated urea, N_2O emissions even increased in the Andosol and were ineffective in the Fluvisol. The authors concluded that varying activities in the main N_2O production processes, nitrification and denitrification, in the different soils were responsible for the contrasting results of the EEFs [45].

2.3. Organic amendments

The role of OAs in altering N_2O emissions is problematic [15, 20]. Organic amendments directly contribute to N_2O emissions through N transformation processes. OAs also indirectly influence N_2O emissions, e.g., through altering the soil pH or through their C:N ratio. A high C:N ratio increases soil organic matter; however, a low C:N ratio may increase N_2O emissions from soils [20–22, 47].

The results concerning N_2O emissions after OA application are contradictory and also depend on which OA is applied. Some studies report reduced or similar N_2O emissions after OA application when compared to conventional fertilizers. Ref. [19] tested the effects of composted pig manure compared to a conventional fertilizer (ammonium nitrate) on N_2O emissions.

After 7 years of applying both fertilizers, they found (14 months after the last application) that denitrification rates were higher in the pig compost treatment probably due to higher organic C contents and microbial biomass. However, N_2O emissions were lower, possibly due to high organic C contents and higher pH which favors N_2 production. The authors concluded that even though denitrifier activity increased under pig compost, it is still a vital option to reduce N_2O emissions if the conditions are more favorable for N_2 production.

However, OA application can also increase N_2O emissions through increased denitrification rates. The decreases in soil pH and the subsequent enhanced microbial respiration lead to a decrease in the redox potential which provides good conditions for denitrifiers [20, 21]. Other factors favoring denitrification are increased soil moisture (which leads to decreasing O_2 concentrations) and using residues with a high N content and low C/N ratios. In a 28 day study by [21], different residues (leguminous species and ryegrass) were incorporated into loamy sand soil with different soil moisture levels. The authors found that N_2O emissions mainly derived from denitrification and were stimulated after residue application in all treatments. However, the level of emissions strongly depended on soil moisture and residue quality. The incorporation of legumes, which are characterized by high N contents and low C/N ratios, induced faster and greater emissions than ryegrass and control soil. Thus, higher soil moisture and residues with a low C/N ratio like lettuce or clover can lead to higher N_2O emissions [21, 23].

Especially when applied to soils with low C content, animal manure and slurry tend to increase N_2O emissions because of their large readily available C and N concentrations. The newly available C leads to a sudden rise in microbial activity and O_2 consumption which creates anaerobic conditions favorable for denitrification [20].

A meta-study that analyzed crop residue effects on N_2O emissions found no statistically significant effect on N_2O emissions when averaged across all studies [22]. They concluded that the quantity of N_2O emissions from OAs like residues depends on many factors including synthetic N fertilizer application, residue type, and application method, as well as land use type. For example, compared to controls, N_2O emissions were decreased by 12% when applied together with synthetic fertilizers but increased by 42% if only residues were applied. The combination of chemical fertilizer with crop residues seems to inhibit N_2O emissions through decreased dissolved organic carbon (DOC) in the soil, thus decreasing denitrification rates. N_2O emissions highly depended on land use type and were decreased by 27% in paddy fields but increased by 93 and 24% in fallows and uplands, respectively. For upland soil, the application of residues led to favorable (increased) water and soil temperature conditions for microbes related to N_2O emissions. The application method can also influence N_2O emissions. For example, mulching of crop residues, which seems to enhance microbial activity, increased emissions by 63%, while ditch mulching, burying, and burning all decreased emissions by around 27%. Also, lettuce and bean residues enhanced N_2O emissions by 123 and 138%, respectively, probably due to their low C/N ratios of 7.5 and 12, respectively [22]. An incubation study that assessed the effects on N_2O emissions of a wide range of C/N ratios of plant residues with or without urea also found that lower C/N ratios led to higher DOC concentrations which increased N_2O emissions. They also found that the residue treatments increased, while residues + urea decreased DOC which affected N_2O emissions [47].

More studies are necessary to evaluate the effectiveness of OAs in reducing N_2O emissions. OA application mainly affects soil parameters like moisture, pH, O_2 concentration, and substrate availability; however, these factors are the main influencers of nitrification and denitrification processes. Possibilities to influence and mitigate N_2O emissions after OA application mainly arise from changing the management practices, e.g., cultivation practices and application rates. To avoid NH_3 volatilization, OAs should be incorporated into the soil to avoid contact with the atmosphere; however, this might increase N_2O emissions due to higher N availability in the soil [20].

A possible option to mitigate N_2O emissions from OAs is to combine them with EEFs. In combination with NIs, N_2O emissions from liquid manure like cattle slurry or pig manure can be greatly reduced [15, 48–50]. [48] tested the effect of the NI DMPP in reducing N_2O emissions from grassland after cattle slurry application. They found that DMPP reduced cumulative N_2O emissions by 69 and 48% in autumn and spring, respectively. In another study using pig manure on irrigated rice in a Mediterranean environment, DMPP also reduced N_2O emissions [50]. But so far, studies on the combination of OAs with NIs are scarce compared to the single treatments.

In summary, OAs have the potential to be environmental-friendly if soil parameters like moisture and pH are carefully evaluated, and the best management practices, e.g., no tillage or split application, are applied. In addition, careful assessment of optimal OA rates, improved timing of OA application, and crop N demand or using a combination of OAs and stabilized fertilizers are some of the options to decrease N_2O emissions after OA application [20].

3. Conclusion and recommendations

Overall, the N budget has to be understood first to estimate the potential benefit of EEF on N_2O emissions. N_2O emission peaks and the annual contribution of different periods (e.g., sowing, after harvest, rainy season) have to be assessed to determine the use of EEF effectively. Also, the contribution of nitrification, denitrification, and other processes concerning N turnover needs to be assessed in more detail. Especially, microbes, the drivers of these processes, need to be addressed in terms of changes in their activity, community structure, and abundance after EEF application and the subsequent changes in N turnover processes. In summary, more studies are necessary to assess the overall effectiveness of EEFs since their power in mitigating N_2O emissions varies with many factors. An individual fertilizer plan and type which is optimized for the prevalent climate conditions, soil type, management practice, etc. would be optimal in order to effectively reduce N_2O emissions. The use of EEFs has to be combined with the assessment of an N balance approach to fully exploit their potential.

Conflict of interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interests; and expert testimony or patent-licensing arrangements) or nonfinancial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

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Management of Paddy Soil towards Low Greenhouse Gas Emissions and Sustainable Rice Production in the Changing Climatic Conditions

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Abstract

Climate change is a vital environmental issue for the twenty-first century, which may significantly affect rice productivity and accelerate greenhouse gas emissions from paddy ecosystem, which is of great environmental concern which is of great environmental concern. Methane (CH_4) and nitrous oxide (N_2O) are the most important greenhouse gases due to their radiative effects as well as global warming potentials (GWPs). CH_4 and N_2O gases are simultaneously emitted from rice fields to the atmosphere due to their favorable production, consumption, and transport systems. The intensive rice farming system has been creating excessive pressure on rice fields to produce more rice for the expanding world population, thereby deteriorating soil fertility status and rice paddy ecosystem balance by stimulating more CO_2, CH_4, and N_2O fluxes to the atmosphere. The extreme climatic variables such as high light intensity, high water vapor or relative humidity, high temperature, and drought stress may badly suppress beneficial microbial activity, soil nutrients, and water availability to rice plant; eventually, rice yield may be decreased drastically, and simultaneously, greenhouse gas emissions could be increased significantly. In this situation, conservation tillage, water saving irrigation technique such as alternate wetting and drying, soil amendments with biochar, vermicompost, azolla-cyanobacterial mixture, recommended silicate slag, and phospho-gypsum with minimum NPKSZn fertilizer (IPNS) should be introduced to the field level farmers for sustainable rice production and mitigating greenhouse gas emissions.

Keywords: paddy soils, greenhouse gases, climate change, rice yield, GWPs

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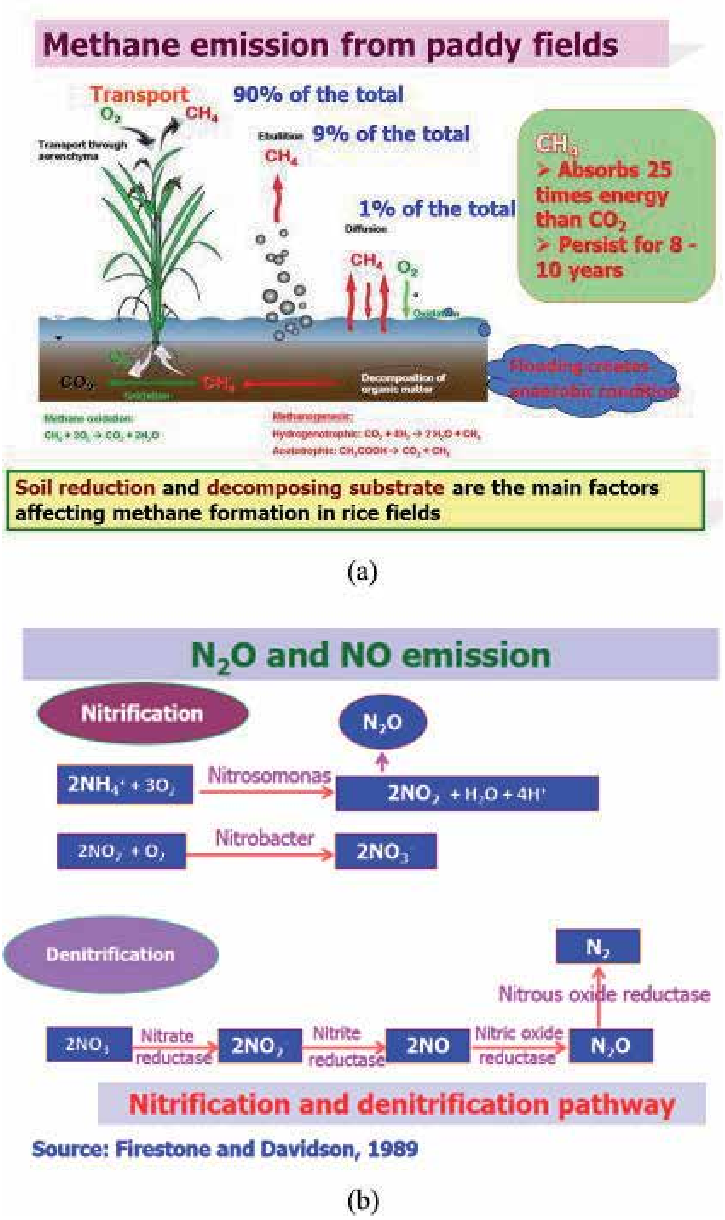
1. Introduction

World rice production especially in Southeast Asia and tropical Asia is highly vulnerable to climate change. Rice production systems contribute to global climate change through emissions of carbon dioxide (CO_2), methane (CH_4), and N_2O gases to the atmosphere and simultaneously



(a)

(b)

Figure 1. (a) Schematic diagram of methane production, oxidation, and emission from rice paddy field and (b) schematic diagram of N_2O, NO, and N_2 emissions from rice paddy field.

are affected by the changed climatic variables. Rice is the major cereal crop for more than half of the world's population and its production needs to be increased 40% by the end of the 2030s to meet the increasing demand for the expanding population [1], which may further accelerate CH_4 and N_2O emissions to the atmosphere [2]. In 2012, worldwide rice production covered 163 million ha of cropland, where approximately 80 million ha were managed under continuous flood irrigation and contributed to 75% of the world's rice production [3]. China and India, most densely populated countries in the world, account for 20.0 and 28.5% of the global rice area, respectively [4]. In China, approximately 90% of the rice fields are irrigated [5], while in India, more than 46% of the rice fields are irrigated [6]. Unfortunately, the irrigated rice farming acts as one of the main sources of anthropogenic CH_4 emission to the atmosphere [7]. Therefore, IRRI is promoting water-saving alternate wetting and drying techniques for improving water use efficiency, while reducing CH_4 production in the rice rhizosphere.

Rice paddy fields act as a source of greenhouse gases such as methane (CH_4) and nitrous oxides (N_2O) depending on soil organic matter status, land use and cropping intensity, irrigation water and drainage management practices, soil microbial populations and their activities, soil properties, and climatic variables. The management practices such as tillage operations, leveling, plant residue incorporation, irrigation frequency and standing water levels, drainage system, and organic and inorganic soil amendments followed in rice farming influence the amount of CH_4 and N_2O emitted to the atmosphere. Generally, CH_4 gas is produced under flooded or anoxic soil conditions (**Figure 1a**), while N_2O gas is produced through nitrification and denitrification processes depending on soil aerobic (oxygenated) and anaerobic conditions (**Figure 1b**).

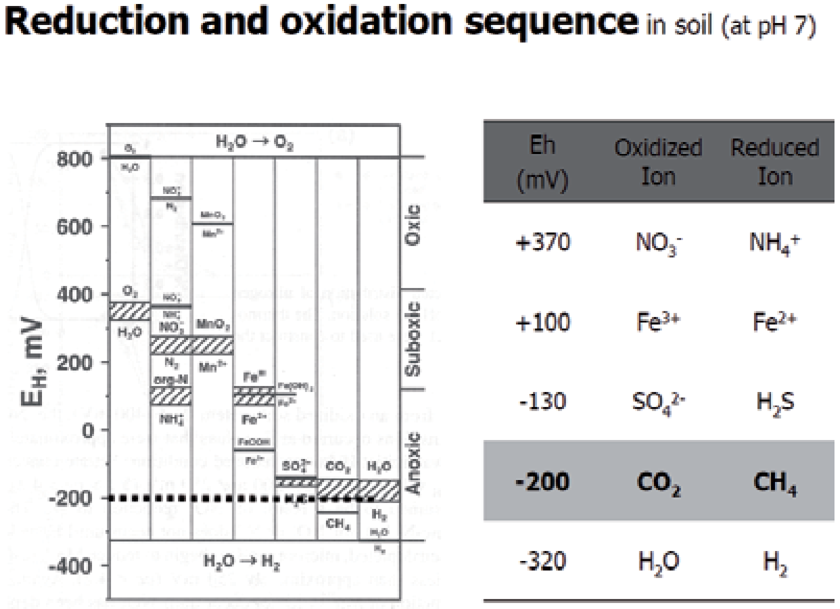
Typically, N_2O emissions are low under flooded fields, while CH_4 emissions are high, a trade-off relationship observed which is largely dependent on paddy soil water level, redox status, soil organic matter content, and external sources of organic and inorganic soil amendments. Ali et al. reported that the total GWP of CH_4 and N_2O gases was decreased by 7–27% and 6–34% with calcium carbide, phospho-gypsum, and silicate fertilizer amendments under continuous and intermittent irrigations, respectively [8]. However, biochar amendments increased the overall GWP of CH_4 and N_2O gases, which simultaneously increased rice yield [8].

2. Climatic change and greenhouse gas emissions

Greenhouse gases (GHGs), mainly carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), have been contributing to about 80% to the current global radiative forcing [9]. Agricultural activities contribute to approximately 20% of the present concentrations of atmospheric GHGs [10], especially the emissions of CH_4 and N_2O from paddy fields [9]. Methane (CH_4) and nitrous oxide (N_2O) are the two most important GHGs from agriculture, with global-warming potentials (GWP) of 25 and 298 CO_2-equivalents, respectively, on a 100-year time horizon. Apart from the water vapor, CH_4 is a major greenhouse gas contributing 20% toward global warming with almost 25-fold higher global warming potential than carbon dioxide [11]. The concentrations of atmospheric CH_4 and N_2O have increased from 722 and 270 ppb in the pre-industrial period to 1853 and 328.9 ppb in 2016, respectively [12]. China, the largest rice-producing country, accounts for about 28% of global rice production [4] and the total CH_4 and N_2O emissions from paddy fields are estimated to be 6.4 $Tgyr^{-1}$ and 180 $Ggyr^{-1}$, respectively [13]. Although the global estimates of CH_4 emission from rice cultivation vary within 20–150 $TgCH_4$ $year^{-1}$, the global average CH_4 is about 100 $TgCH_4$ $year^{-1}$ [14] and may increase further due to the expansion of rice cultivation as well as intensification of rice agriculture for the increasing world population [15]. Therefore, it is very important to understand the mechanism of CO_2, CH_4, and N_2O exchange and their main controlling factors for developing appropriate strategies to mitigate GHG emissions.

3. Characteristics of paddy soils

Paddy soils are mostly alluvial soils and low humic gley soils (or Entisols and Inceptisols). In addition, vertisols, reddish-brown earths or Alfisols, red-yellow podzolic soils or Ultisols, and latosols or Oxisols are utilized for paddy rice cultivation. Paddy soils are found mainly on alluvial lands such as deltas and flood plains of big rivers, coastal plains, fans, and lower terraces. In general, paddy soils are resistant to erosion when they are terraced and there are ridges around the field, as measures to retain surface water. Paddy fields in the lowlands receive new sediments deposited from run-off that carries eroded topsoil down from the uplands, thus sustaining soil fertility and productivity. The paddy soils have medium to high organic matter (1.5–3.97 g/kg), available phosphorus (11.7–19.9 mg/kg), available potassium (61.6–132.9 mg/kg), and cation exchange capacity (15.5–33.1 cmol/kg). The most common practice in paddy rice cultivation is flooding or temporary water logging of the land surface. Soil redox potential (Eh) or electron activity in soil gradually decreases after flooding, which causes significant methane production at around −200 mV [16], and creates high risk of gaseous N losses through denitrification (**Figure 2**).



Eh (mV)	Oxidized Ion	Reduced Ion
+370	NO_3^-	NH_4^+
+100	Fe^{3+}	Fe^{2+}
-130	SO_4^{2-}	H_2S
-200	**CO_2**	**CH_4**
-320	H_2O	H_2

Figure 2. Paddy soil redox status, sequential reduction and oxidation of inorganic nitrogen, manganese, and iron in flooded soil, and methane gas formation [16].

In paddy fields, the kinetics of the reduction processes are strongly affected by the composition and texture of soil and its content of inorganic electron acceptors. After flooding, microbial reduction processes sequentially use NO_3^-, Mn^{4+}, Fe^{3+}, and SO_4^{2-} as electron acceptors, accompanied by the emission of the trace gases N_2O, N_2, H_2S, and CH_4 due to reduction-induced increasing pH-NH_3 (**Figure 2**). Microorganisms drive redox reactions in soil by using organic carbon and electron acceptors for their metabolic activities. Methane is produced at the terminal step under anaerobic decomposition of organic matter and due to the reduction of CO_2 into CH_4 in wetland soils. Soil Eh values decreased rapidly after flooding within 5–7 weeks then stabilized toward −200 to −240 mV and produced significant amount of methane [17]. High concentrations and fluxes of dissolved organic matter (DOM) in paddy soils from plant debris trigger microbial activity and thus the emission of greenhouse gases. Therefore, the objectives of this thematic topic are to highlight the feasible field management practices for sustainable rice production and recommend appropriate strategies to mitigate GHG emissions from paddy soils in the changing climate.

4. Materials and methods

4.1. Measurement of CH_4 and N_2O emissions from the paddy field

The static closed chamber technique was used to measure CH_4 and N_2O emissions during rice growing period [18]. The chambers were made of PVC and consisted of two parts: an upper transparent compartment (100 cm height, 30 cm width, and 30 cm length) was placed on a permanently installed bottom collar (10 cm height, 30 cm width, and 30 cm length). Three replicate chambers were used. Each of these chambers was placed in each plot. Each chamber was installed with a battery-operated fan to homogenize the air inside the chamber headspace, a thermometer to monitor temperature changes during the gas-sampling period, and a gas-sampling port with a neoprene rubber septum at the top of the chamber for collecting gas samples from the headspace. Gas samples were collected twice daily (**Figure 3**), sampling during 9.00 am to 12.00 and 12.30 pm to 3.30 pm. A 100-mL plastic syringe equipped with a 3-way stopcock was used to collect gas samples from the chamber headspace 0, 15, and 30 min after chamber deployment. Gas samples were collected twice a day. The collected gas samples were immediately transferred to 100-mL air-evacuated aluminum foil bags (Delin Gas Packaging Co., Ltd., Dalian, China) sealed with a butyl rubber septum and transported to the laboratory for analysis of CH_4 and N_2O concentrations by Gas Chromatograph (**Figure 4**).

4.2. Determination of CH_4 and N_2O concentrations in the headspace air samples

CH_4 and N_2O concentrations in the headspace air samples were determined by a gas chromatograph (Shimadzu GC-2014, Kyoto, Japan) packed with a Porapak Q column (2 m length, 4 mm OD, 80/100 mesh, stainless steel column) (**Figure 4**). A flame ionization detector (FID) and an electron capture detector (ECD) were used for the determination of CH_4 and N_2O concentrations, respectively. Helium (99.9% purity) was used as a carrier gas (30 ml min^{-1}), and a make-up gas (95% argon and 5% CH_4) was used for the ECD. Calibration was conducted with 1.01, 7.99, and 50.5 μl CH_4 l^{-1} in He and 0.2, 0.6, and 1.0 μl N_2O l^{-1} in He (CRM/RM Information Center of China) as primary standards.

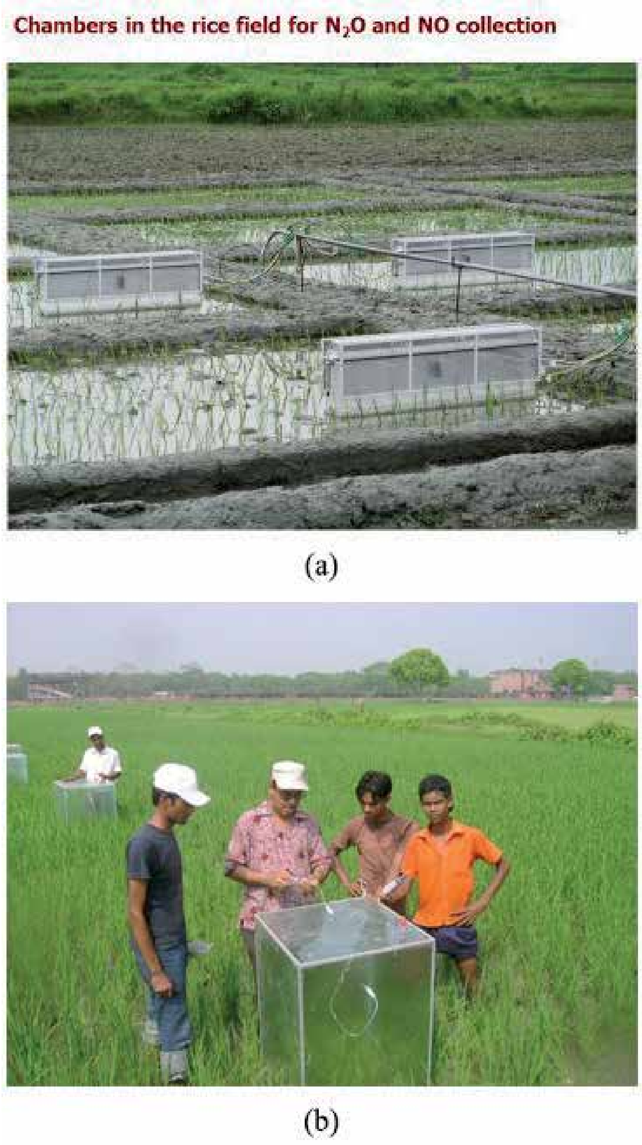


Figure 3. (A) Prototype and (B) gas sampling in rice planted paddy field through the closed chamber technique [18].

4.3. Estimation of CH_4 and N_2O fluxes, GWPs, and greenhouse gas intensity (GHGI)

CH_4 and N_2O fluxes from the paddy field were expressed as the increase/decrease in CH_4 and N_2O mass per unit surface area per unit time. CH_4 and N_2O fluxes were estimated by the following equation [19]: $F = MV*dc/dt*H*(273/(273 + T)$; where F is the CH_4 or N_2O flux (mg CH_4 m^{-2} h^{-1} or $\mu g N_2O$ m^{-2} h^{-1}), M is the molar mass of the respective gas (16 for CH_4 and 44 for N_2O), V is the molar volume of air at a standard state (22.4 l mol^{-1}), dc/dt is the change in headspace CH_4 and N_2O concentration with time (μmolmol h^{-1}), H is the height of the chamber above the water surface (m), and T is the air temperature inside the chamber (°C).

Figure 4. Injecting air samples into GC for determination of CH_4 and N_2O concentrations.

The total CH_4 or N_2O emission from paddy fields was the summation of methane and nitrous oxide emissions in all growth stages of rice crop.

4.4. Estimation of GWPs of CH_4 and N_2O

To estimate the GWP, CO_2 is typically taken as the reference gas, and an increase or reduction in emission of CH_4 and N_2O is converted into "CO_2-equivalents" by means of their GWPs. In this study, we used the IPCC factors to calculate the combined GWPs for 100 years [GWP = $(25 \times CH_4) + (298 \times N_2O)$], kg CO_2-equivalents ha^{-1} from CH_4 and N_2O under various agricultural practices. In addition, the greenhouse gas intensity (GHGI) was calculated by dividing GWP by grain yield for rice [20, 21].

5. Results and discussion

The mean rice statistics and cumulative methane emissions in the rice producing countries are presented in **Table 1.**

It is known that China, India, Indonesia, Bangladesh, and Vietnam have been playing a dominating role in total rice production (**Table 1**), which may impose a threat to the environment by stimulating CH_4 emissions. Phospho-gypsum, a by-product of the phosphate fertilizer manufacturing industry, is a feasible soil amendment to supplement mainly calcium and sulfur for rice cultivation. The high content of sulfate in phospho-gypsum might prevent CH_4 formation as well as CH_4 emissions due to stronger competitor for substrates (hydrogen or acetate) than methanogens. It was reported that silicate slag and phospho-gypsum in combination with Azolla-cyanobacteria significantly decreased CH_4 flux while improved rice rhizospheric redox status (**Figure 5**) [18, 23]. Ali et al. reported that biochar amendments in paddy soils of Japan and Bangladesh decreased seasonal cumulative N_2O emissions by 31.8 and 20.0%, respectively, followed by 26.3 and 25.0% reduction with biochar plus Azolla-cyanobacteria amendments (**Table 2**) [18]. Seasonal cumulative CH_4 emissions and global warming potentials were significantly decreased due to Azolla-cyanobacterial inoculation with phospho-gypsum and

Country	Harvested area (Mha)	Total production (Mt)	Yield (kg ha^{-1})	CH_4 emission(kg ha^{-1})
India	43	146	43,400	110.1
China	29	195	6500	180
Indonesia	12	63	4900	210
Bangladesh	11	46	4200	100
Vietnam	7	40	5300	180
Brazil	2	12	44,300	60
Colombia	0.5	2	4600	210
Peru	0.4	3	7200	240
Argentina	0.2	1	6600	280
Uruguay	0.2	1	7600	280

Source: M indicates million [22].

Table 1. Mean rice statistics (2004–2014) in the main rice producer countries in Asia, Latin America, and the Caribbean (LAC).

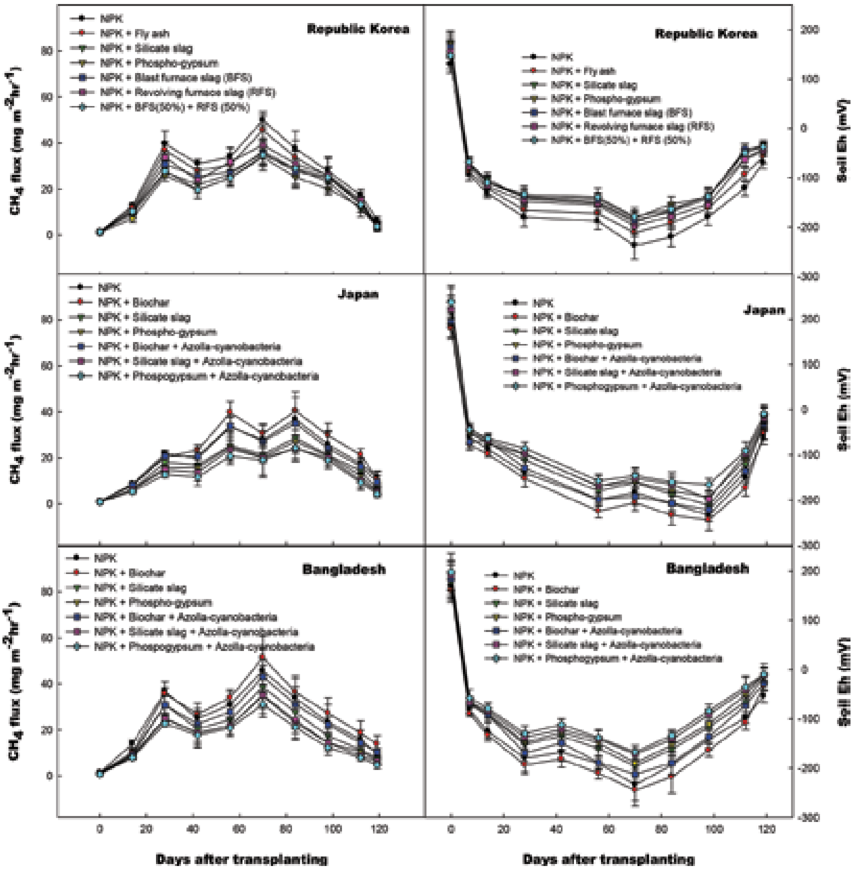


Figure 5. Trends of CH_4 flux and soil Eh with different soil amendments during rice cultivation in Bangladesh, Japan, and Korea [18, 23].

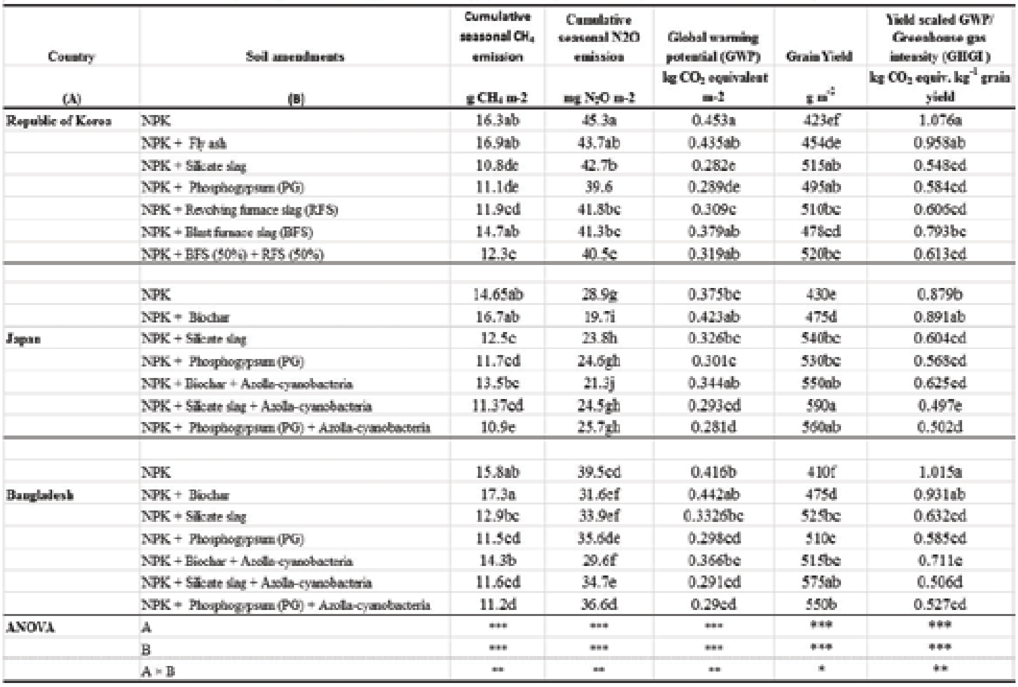
silicate slag amendments [18]. Combined effects of blast furnace slag and revolving furnace slag amendments also showed decreasing effects on GWPs (**Table 2**).

Silicate slag and biochar amendments in different soils of Japan also reduced cumulative CH_4 flux, while increased rice growth and yield parameters (**Table 3**) [23].

Among the amendments, biochar significantly decreased N_2O emission rates (**Figure 6**); however, it increased CH_4 emission rates (**Figure 1**). Silicate slag and phospho-gypsum amendments lowered N_2O emission rates compared to control treatment (NPK), although no significant differences were observed (**Figure 6**) [18].

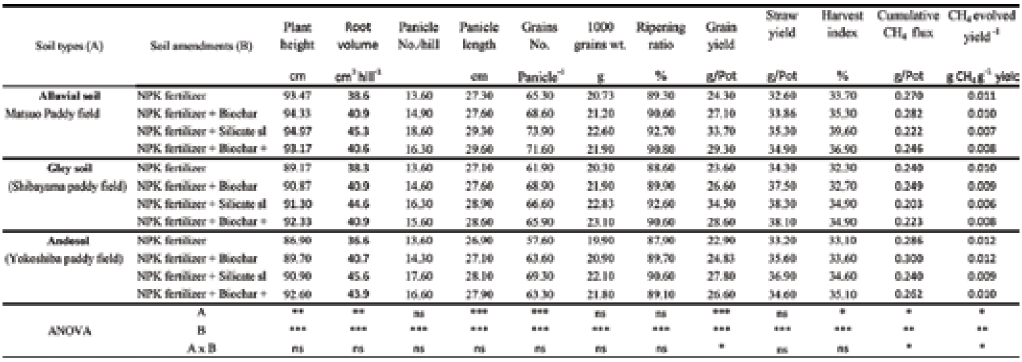
5.1. Climate change and threats to rice production

The IPCC 4th Assessment Report (IPPC) states that Southeast Asia is expected to be seriously affected by the adverse impacts of climate change [11]. The frequency of floods, drought, cyclones, tornadoes, thunderstorm, and earthquake increased during the last 5 years, which badly affected the natural vegetation and forest covers, wild animals, wetlands, and land resources, and ultimately, agricultural productivity declined. In Indonesia, the Philippines, Thailand, and Vietnam, the annual mean temperatures are projected to rise by 4.8°C by 2100, and the global mean sea level will increase by 70 cm during the same period [24]. It has been reported that in Southeast Asia, small changes in the annual rainfall are expected to continue up to 2040 [25], and there will be an increase in the occurrence of severe weather including heat waves and precipitation events. Increases in tropical cyclone intensities by 10–20% are



Country	Soil amendments	Cumulative seasonal CH_4 emission	Cumulative seasonal N2O emission	Global warming potential (GWP)	Grain Yield	Yield scaled GWP/ Greenhouse gas intensity (GHGI)
(A)	(B)	g CH_4 m-2	mg N_2O m-2	kg CO_2 equivalent m-2	g m^{-2}	kg CO_2 equiv. kg^{-1} grain yield
Republic of Korea	NPK	16.3ab	45.3a	0.453a	423ef	1.076a
	NPK + Fly ash	16.9ab	43.7ab	0.435ab	454de	0.958ab
	NPK + Silicate slag	10.8de	42.7b	0.282e	515ab	0.548cd
	NPK + Phosphogypsum (PG)	11.1de	39.6	0.289de	495ab	0.584cd
	NPK + Revolving furnace slag (RFS)	11.9cd	41.8bc	0.309c	510bc	0.606cd
	NPK + Blast furnace slag (BFS)	14.7ab	41.3bc	0.379ab	478cd	0.793bc
	NPK + BFS (50%) + RFS (50%)	12.3c	40.5c	0.319ab	520bc	0.613cd
	NPK	14.65ab	28.9g	0.375bc	430e	0.879b
	NPK + Biochar	16.7ab	19.7i	0.423ab	475d	0.891ab
Japan	NPK + Silicate slag	12.5c	23.8h	0.326bc	540bc	0.604cd
	NPK + Phosphogypsum (PG)	11.7cd	24.6gh	0.301c	530bc	0.568cd
	NPK + Biochar + Azolla-cyanobacteria	13.5bc	21.3j	0.344ab	550ab	0.625cd
	NPK + Silicate slag + Azolla-cyanobacteria	11.37cd	24.5gh	0.293cd	590a	0.497e
	NPK + Phosphogypsum (PG) + Azolla-cyanobacteria	10.9e	25.7gh	0.281d	560ab	0.502d
	NPK	15.8ab	39.5cd	0.416b	410f	1.015a
Bangladesh	NPK + Biochar	17.3a	31.6ef	0.442ab	475d	0.931ab
	NPK + Silicate slag	12.9bc	33.9ef	0.3326bc	525bc	0.632cd
	NPK + Phosphogypsum (PG)	11.5cd	35.6de	0.298cd	510c	0.585cd
	NPK + Biochar + Azolla-cyanobacteria	14.3b	29.6f	0.366bc	515bc	0.711c
	NPK + Silicate slag + Azolla-cyanobacteria	11.6cd	34.7e	0.291cd	575ab	0.506d
	NPK + Phosphogypsum (PG) + Azolla-cyanobacteria	11.2d	36.6d	0.29cd	550b	0.527cd
ANOVA	A	***	***	***	***	***
	B	***	***	***	***	***
	A × B	**	**	**	*	**

Table 2. Cumulative seasonal CH_4 and N_2O emissions, global warming potentials (GWPs), and yield scaled GHG intensity under different soil amendments. Source: [18, 23].



Soil types (A)	Soil amendments (B)	Plant height	Root volume	Panicle No./hill	Panicle length	Grains No.	1000 grains wt.	Ripening ratio	Grain yield	Straw yield	Harvest index	Cumulative CH_4 flux	CH_4 evolved yield^{-1}
		cm	$cm^3 hill^{-1}$		cm	$Panicle^{-1}$	g	%	g/Pot	g/Pot	%	g/Pot	g CH_4 g^{-1} yielc
Alluvial soil	NPK fertilizer	93.47	38.6	13.60	27.30	65.30	20.73	89.30	24.30	32.60	33.70	0.279	0.011
Matsuo Paddy field	NPK fertilizer + Biochar	94.33	40.9	14.90	27.60	68.60	21.20	90.60	27.10	33.86	35.30	0.282	0.010
	NPK fertilizer + Silicate sl	94.97	45.3	18.60	29.30	73.90	22.60	92.70	33.70	35.30	39.60	0.222	0.007
	NPK fertilizer + Biochar +	93.17	40.6	16.30	29.60	71.60	21.90	90.80	29.30	34.90	36.90	0.246	0.008
Gley soil	NPK fertilizer	89.17	38.3	13.60	27.10	61.90	20.30	88.60	23.60	34.30	32.30	0.240	0.010
(Shibayama paddy field)	NPK fertilizer + Biochar	90.87	40.9	14.60	27.60	68.90	21.90	89.90	26.60	37.50	32.70	0.249	0.009
	NPK fertilizer + Silicate sl	91.30	44.6	16.30	28.90	66.60	22.83	92.60	34.50	38.30	34.90	0.203	0.006
	NPK fertilizer + Biochar +	92.33	40.9	15.60	28.60	65.90	23.10	90.60	28.60	38.10	34.90	0.223	0.008
Andosol	NPK fertilizer	86.90	36.6	13.60	26.90	57.60	19.90	87.90	22.90	33.20	33.10	0.286	0.012
(Yokoshiba paddy field)	NPK fertilizer + Biochar	89.70	40.7	14.30	27.10	63.60	20.90	89.70	24.83	35.60	33.60	0.309	0.012
	NPK fertilizer + Silicate sl	90.90	45.6	17.60	28.10	69.30	22.10	90.60	27.80	36.90	34.60	0.240	0.009
	NPK fertilizer + Biochar +	92.60	43.9	16.60	27.90	63.30	21.80	89.10	26.60	34.60	35.10	0.262	0.010
	A	**	**	ns	***	***	ns	ns	***	ns	*	*	*
ANOVA	B	***	***	***	***	***	***	***	***	***	***	**	**
	A x B	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	*	*

Table 3. Rice plant growth, yield components, and cumulative CH_4 flux under biochar and silicate amendments in different field sites of Japan. Source: JSPS Report by Ali [23].

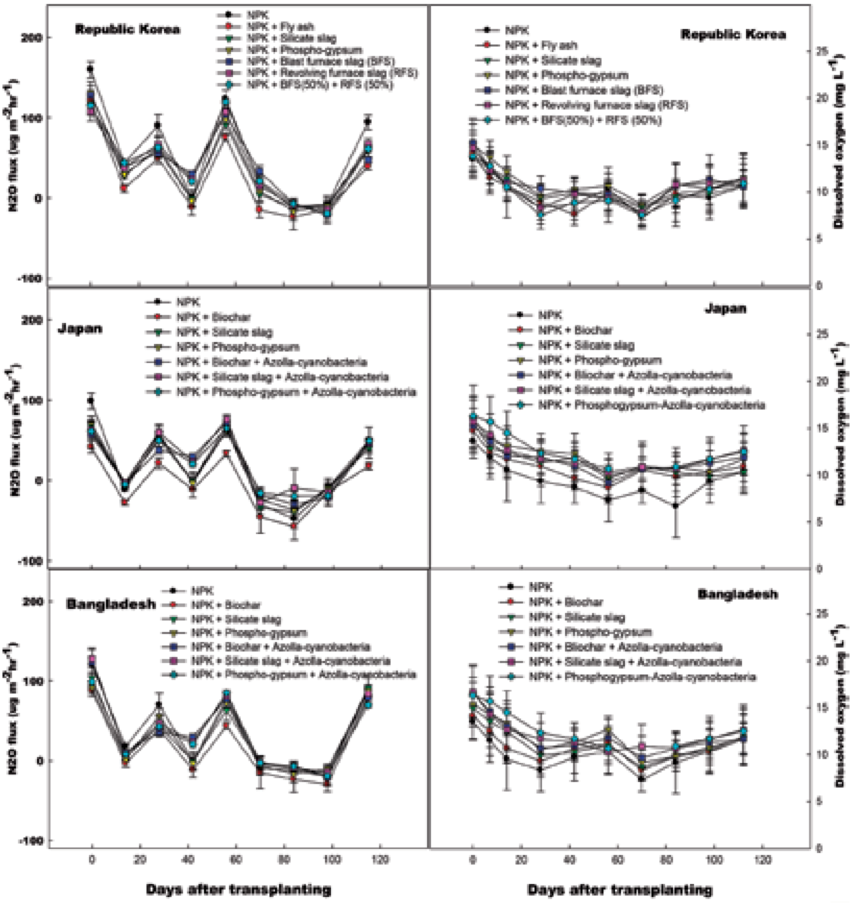


Figure 6. Trends of N_2O flux and DO concentrations under different soil amendments during rice cultivation in Bangladesh, Japan, and Korea [18].

Treatments	Flood water paddy field (Showair Union)						Flood water paddy field (Tetulia Union)					
	Grain yield (kg/ha)	Straw yield (kg/ha)	Gross return (Tk./ha)	Total variable cost (Tk./ha)	Net return (Tk./ha)	BCR	Grain yield (kg/ha)	Straw yield (kg/ha)	Gross return (Tk./ha)	Total variable cost (Tk./ha)	Net return Tk./ha	BCR
Control (no top dressing, no ducklings)	2143	4146	44,940	30,333	14,606	1.48	2106	4126	44,196	30,266	13,930	1.46
NPKS (100%) + ducklings	2576	5106	104,086	49,450	54,636	2.12	2496	4840	100,353	49,450	50,903	2.04
NPKS (50%) + bioslurry with oyster shell + ducklings	2446	4850	101,358	49,750	51,608	2.04	2433	4883	99,108	49,750	49,558	2.0
NPKS (50%) + vermicompost + ducklings	2776	5516	108,291	50,733	57,558	2.14	2693	5370	104,551	47,400	57,151	2.20
NPKS (50%) + azolla-cyanobacterial mixture + duckling	2650	5283	105,641	48,333	57,308	2.20	2603	5183	102,658	48,334	54,325	2.14
Significance level	**	**	**	*	*	*	*	*	*	*	*	*

Table 4. Overall productivity of rice duck farming in wetland paddy ecosystem of Dingaputa Haor, Netrokona district by Ali [28].

anticipated, and temperatures are projected to continue to increase by about 0.7–0.9°C [25]. Furthermore, sea levels have risen by 1–3 mm/year, marginally higher than the global average [24]. Rice production systems of this region have become increasingly threatened by the effects of climate change as a large portion of the rice-growing areas are located in especially vulnerable regions. A decrease of 10% in rice yield has been found to be associated with every 1°C increase in temperature [24], while the yield of dry-season rice crops in the Philippines decreased by as much as 15% for each 1°C increase in the growing season mean temperature. These temperature and aggravating climate change effects may cause a decline in the world rice production, which have already shown negative effects on agricultural production. By 2100, Indonesia, the Philippines, Thailand, and Vietnam are projected to experience a potential fall of about 50% in rice yield due to the occurrence of extreme climatic events [24, 26]. Furthermore, rice yield would be affected severely due to sea level rise and intrusion of saline water in the coastal area, which will hamper rice growth and yield. Rising sea levels in association with heavy monsoon rainfall will create serious waterlogging and prolonged stagnant floods in major rice-growing, low lying mega-deltas in Southeast Asia, which ultimately deteriorate rice production in the deltas since only a few low-yielding rice varieties have evolved to withstand such conditions [27]. The recent natural disasters such as flash floods caused by water of Indian Meghalaya state and excessive rainfall in the low lying ha or areas of Bangladesh [28] badly affected the only cultivated rice crop, the Boro rice (winter rice), at this region; however, due to rice duck mixed farming, the overall productivity and net profit were recovered to some extent (**Table 4**).

6. Management of rice paddy ecosystem to cope with climate change and sustainable rice production

6.1. Soil conservation with suitable cover crops and minimum tillage or no tillage

Soil conservation practices such as suitable cover crops, mulches, and minimum tillage (one or two plowing with proper leveling) may be introduced in rice farming not only to control soil erosion and land degradation, while reduce production costs to sustain rice productivity. In addition, conservation tillage will improve environmental quality by lowering GHG emissions (less air pollution) through decreasing the use of diesel fuel and nonburning of rice residues [29]. It has also reported that the no-tillage system in Korean paddy field with silicate fertilization decreased total seasonal CH_4 flux by 53 and 36%, while maximizing grain yield by 18 and 13% over the control tillage and control no-tillage systems, respectively [30]. Soil properties were also improved with silicate fertilization under the no-tillage system. It was [31] reported that tillage (after the harvest of late rice) with the incorporation of stubble (3.5 t ha^{-1}) in the winter fallow season significantly decreased both the net GWP and the GHGI while maintained a high grain yield (13.0–13.3 t ha^{-1} yr^{-1}) in the double-cropping rice system.

6.2. Introducing direct seeded rice (DSR) and puddle rice transplanting (PRT) methods

Direct seeded rice (DSR) is a process of establishing a rice crop which is done by seeds sown in the field rather than by transplanting seedlings from the nursery. The practice of direct seeding instead of transplanting resulted in a 16–54% reduction in CH_4 emission [32]. CH_4 emission was more significantly reduced under dry-direct seeding compared to wet-direct seeding. However, grain yield in direct seeded rice (DSR) was found lower than Puddle transplanted rice (PTR), probably due to poor crop stand, high percentage of panicle sterility, and higher weed and root-knot nematode infestation [33]. It was also observed that grain yield of direct-seeded rice (9.0 Mg ha^{-1}) was identical to grain yield of transplanted-flooded rice [34]. Average yield penalty of around 10% was observed for the direct seeded rice (DSR) compared with puddle transplanted rice [34]. It was reported [35] that over the rice-growing season, cumulative CH_4 emissions were significantly higher in puddle transplanted rice compared to the direct seeded rice production system.

6.3. Water management for sustainable rice production and minimizing GHG emissions

Water management influences rice yield and CH_4 and N_2O emissions from rice cultivation systems. Irrigated rice fields are an integral part of the rice production system in Asian countries, which contribute about 75% to global rice production. Single or multiple drainages during a rice growing season (e.g., AWD) are reported to reduce CH_4 emissions by 48–93% compared to those observed under continuous flooding systems [36, 37]. Mid-season drainage and intermittent flooding were found effective for increasing productivity and quality of rice as well as reducing methane emissions in Japan [38]. The AWD field showed the same yield as continuous flooded field, but saved 16–24% in water costs and 20–25% in production costs. Most farmers in China, Japan, and South Korea have been practicing this mid-season drainage (5–7 days dry out) to increase rice yield and decrease GHG emissions. Mid-season drainage and intermittent irrigations may reduce methane emissions by about 50%. It was also reported [39] that the AWDI treatment (irrigation applied when water level in the pipe fell 15 cm) showed superiority for the rice yield performance and seasonal CH_4 emission reduction, water savings, and maximum water productivity index. However, the AWD irrigation technique increased the N_2O emission by 97%, especially in DS [40].

6.4. Diverse farm management practices, soil amendments, and rice cropping systems

Feasible management approaches based on agroecosystem have to be adopted to sustain agricultural productivity in the changing climatic conditions. For example, the ground cover rice production system (GCRPS), through which paddy soils are covered by thin plastic films to conserve soil moisture nearly at saturated status, is a promising technology to increase yields with less irrigation water. However, increased soil aeration and temperature under GCRPS may cause more CH_4 to N_2O emissions compared to conventional techniques. Yao et al. [41] reported that the GHG emissions for the ground cover rice production system (GCRPS, i.e., paddy soils being covered by thin plastic film) were found significantly lower (1973 kg CO_2 eq ha^{-1}) than that of traditional cultivation (4186 kg CO_2 eq ha^{-1}). Total seasonal CH_4 emissions under GCRPS were on average 80% lower as compared to the traditional rice cultivation. The yield-scaled GHG emissions from GCRPS were further reduced from 377 to 222 kg CO_2 eq Mg^{-1} as N_2O emissions greatly decreased while yields increased. The system of rice intensification (SRI), an agro-ecological methodology, could be a feasible technique to sustain rice productivity by changing the management of plants, soil, water, and nutrients. Successful application of SRI of increased paddy yield by 50–100% while using less inputs, in particular water, (farmers were able to reduce their water requirements by about 25–50%) [42] has already been reported. Suitable rice cropping patterns, rotations, and mixed rice-duck-fish farming hold the potential scope to sustain agricultural productivity and controlling GHG emissions in the changing climatic conditions. For example, in the Philippines, fish or ducks have been raised with rice as well as legumes such as mung bean (*Vigna radiata*), groundnut (*Arachis hypogaea*), and soybean (*Glycine max*) after two rice dropping. Rotation of crops that have their most drought-sensitive phase in different phases of the growing season may prove a valuable adaptation to limited water resources. Haque et al. reported that the nonrice-based cropping patterns had lower GWPs than the rice-rice-based cropping patterns [43]. Ali et al. reported that CH_4 emissions from wetland paddy ecosystems were significantly decreased by integrated rice duck farming [28]. It has been reported that azolla application in rice field increased CH_4 emission, probably due to the exudation of azolla root and decomposition of dead azolla. In contrast, reverse report on CH_4 emission was also found from rice soil ecosystems, probably due to the increase in redox potential in the root region and dissolved oxygen concentration at the soil-water interface. Azolla cover increased N_2O emission from rice paddies due to N-fixation by azolla providing a source for N_2O production through nitrification and de-nitrification, especially when the azolla died [44]. CH_4 emissions have been reported to increase when crop residues are incorporated prior to planting due to higher amounts of readily available carbon stimulating soil microbial activity. Sander et al. reported that incorporation of rice residues immediately after harvest and subsequent aerobic decomposition of the residues before soil flooding for the next crop reduced CH_4 emissions by 2.5–5 times and also improved nutrient cycling in paddy field [45]. It was also reported that residue incorporation accelerated CH_4 and N_2O emissions from irrigated rice field compared to residues (ryegrass and serradella) left on the soil surface. The open burning of crop residues emits CO_2, CH_4, and N_2O. Ali et al. [17] reported that silicate slag and phospho-gypsum amendments with nitrogenous fertilizer in rice cultivation significantly decreased seasonal CH_4 flux by 16–20% and increased rice yield by 13–18% in Korean paddy soil, whereas 12–21% reduction in total seasonal CH_4 flux and 5–18% increase in rice grain yield were found in the upland rice paddy soils of Bangladesh [46]. Seasonal cumulative CH_4 and N_2O emissions, GWPs, and yield scaled greenhouse gas emissions were decreased by combined application of Azolla-cyanobacterial mixture with silicate slag, phospho-gypsum, and biochar amendments in rice paddy soils of Japan, Korea, and Bangladesh (**Table 2**) [18]. Site-specific nutrient management (SSNM) for rice developed by IRRI (2006) in Asia [47] enables rice farmers to tailor nutrient management to the specific conditions of their fields, and provides a framework for nutrient-based management practices for rice. The increase in annual grain yield with use of SSNM in on-farm evaluation trials averaged 0.9 t/ha in southern India, 0.7 t/ha in the Philippines, and 0.7 t/ha in southern Vietnam [48]. Climatic stress tolerant rice cultivars such as drought, salt/saline, and submergence tolerant rice cultivars have to be developed to cope in the real field stress situation. It was reported [49] that indica-type rice cultivars had significantly higher yield-scaled GWP (1101 kg CO_2 equiv. Mg^{-1}) compared to Japonica (711 kg CO_2 equiv. Mg^{-1})-type rice cultivar. It was also reported that AWD irrigation practice reduced CH_4 emissions by 24–41%, 26–48% compared with continuous flooding, however, an increase in N_2O emission was observed in both seasons [50]. It was also reported that biochar application in paddy soil significantly decreased N_2O emission, while increased CH_4 emission [51].

7. Conclusion

In the context of global climate change, environment friendly agricultural management practices such as conservation tillage, rice seedling transplanting or direct line seeding, alternate wet and dry irrigation (AWDI), mid-season drainage, soil amendments with biochar, vermicompost, silicate slag and phospho-gypsum, site specific rice based cropping patterns and integrated plant nutrients system (IPNS) should be followed to ensure food security, while mitigating greenhouse gas emissions and global warming potentials. Furthermore, Azolla-cyanobacterial dual cropping with rice, introducing N-fixing legumes and duckling rearing with flood water rice cultivation could be practiced to sustain overall agricultural productivity and minimizing greenhouse gases intensity in the changing climatic conditions.

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Chapter 7

Biopesticide of Neem Obtained by Enzyme-Assisted Extraction: An Alternative to Improve the Pest Control

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Additional information is available at the end of the chapter

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Abstract

The indiscriminate use of chemical pesticides to control pests and diseases without technical assistance instead of solving the pest problems has caused environmental damage, agriculture productivity, and human health. Pesticides can remain for several years in the soil, being able to contaminate rivers and lagoons, animals of shepherding and foods. Besides, in recent years, pests have shown an alarmingly resistance over several pesticides. This makes necessary the use of other natural sources of pesticides that could be degraded avoiding the resistance problem. One of the main sources analyzed is the neem (*Azadirachta indica*) due to its complex content of bioactive triterpenoids. However, cellulosic structures of cell wall conditioned the extraction of these components, acting as physical barrier and avoiding its complete extraction. This chapter included a review of the consequences of the use of chemical pesticides to control pests spread in plant and animals and its repercussions on the environment. Moreover, the advantages of the use of food-grade enzyme preparations as an alternative to elaborate an extract of neem without organic solvents are exposed. The results are promissory and could improve the acaricide and repellent effects of the neem extracts over pests, reducing the negative effect caused by chemical pesticides.

Keywords: contamination, pesticide residues, biopesticides, *Azadirachta indica*, food-grade enzymes

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1. Introduction

Pests and diseases have always had repercussions directly on losses of crops and livestock products and indirectly over the income decreases due to insufficient harvests of commercial crops. Chemical pesticides are used in an excessive way and without prior technical assistance to pest distribute control, which instead of solving the problem, has produced strong damage to agricultural and livestock productivity, as well as important environmental effects with implications to public health [1]. Pesticides are chemical substances designed to prevent, delay, repel or fight any pests [2].

In [3], it was mentioned that Mexico ranked fifth in the world in the use of 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (DDT) in agricultural programs and fourth place for its use in public health. As many as 69,545 ton of DDT were used in health campaigns for the control of malaria and agricultural activities, from 1957 onward, DDT was applied every 6 months indoors and outdoors with a coverage of 2 g/m^2, and almost 1000 ton DDT/y were used in agricultural areas [4]. Indeed, DDT was used in Mexico until the year 2000, and DDT and its metabolites have been found in the environment [5] as well as in human tissues [5, 6], breast milk [7], raw cow's milk and bovine meat [8, 9]. DDT is a very stable organochlorine pesticide that is almost completely metabolized, but small percentage remains as o,p′-DDT, while the most of its concentration is transformed into p,p′-DDE, which is characterized for its poor solubility in water and high affinity for lipids. It is considered a pollutant of high persistence due to its half-life of up to 15 years in the environment [10]. This pesticide persistence is responsible of the wild flora and fauna deterioration, as well as the contamination of soil, water table, continental, and coastal waters. Besides, pesticides can be incorporate into pasture, vegetables, and edible animals, which when consumed, act as transporters facilitating its accumulation in living organisms [11, 12].

In the state of Chiapas, México, [13] found high levels of total DDT in outdoor soil samples that ranged from 0.002 to 27 mg kg^{-1}, while the levels found by [4] in Chihuahua, México, ranged from 0.001 to 0.788 mg kg^{-1}. Taking into account the guideline for total DDT in residential soil of 0.7 mg kg^{-1} in Canada [14], the soil samples from Chiapas had levels higher than the guideline. The high levels of OCs observed may be due to ongoing usage as well as the emission of old residues from soil. Soils are an important sink and source for persistent organic pollutants to the atmosphere. In many of the cases, the contamination of soil with pesticides is due to its incorrect storage, either by leakage of corroded tanks containing liquid pesticides or by aerial dissemination of powder pesticide. However, when pesticides infiltrate the soil, their dissemination depends on the nature of the pesticide, as well as the composition, moisture, pH, and temperature [12, 15]. Because of that, a small portion of spilled pesticides can generate a high soil contamination. Moreover, soil pesticides infiltration can cause their introduction and distribution in the food chain, accumulating successively on each ecological niche until reaching lethal doses for some constituent organisms of the chain, or until reaching high levels of the trophic network [16].

This problem is aggravated due to the excessive use of pesticides in the agricultural sector, the absence of technological remediation and the lack of safety interval (waiting period) for the harvest of agricultural products, which has significant impacts on public health [17]. Some of the toxic effects of DDT and its metabolite identified in mammalian have been alterations in fetal development and adverse effects on testicular function (semen, sperm, and sperm motility decrease) due to the mimetization or antagonism of reproductive hormones [18, 19]. Thus, DDT metabolites and DDT are considered endocrine disruptors, with estrogenic properties related to several types of estrogen-dependent cancers, such as breast cancer; therefore, the use of this pesticide was prohibited on the decade of the 1970s in most countries [20]. Nevertheless, pesticides remain in the environment (persistence); therefore, the pesticides have been widely distributed, and their traces can be detected in all areas of the environment (air, water, and soil) [21, 22]; therefore, current tendency is focused on natural sources for biological pesticide control. On the other hand, the indiscriminate and uncontrolled application of synthetic pesticide besides to accumulate residues in the environment and, in some cases, in living beings, has caused resistance in some pest. The first case reported of DDT resistance occurred in 1947, and since then, it has increased alarmingly, and it has been estimated that there are currently around 489 species of pest resistant to 400 different pesticides in the world [23]. The irrational use of synthetic pesticide has produced genotypic and phenotypic changes in many species, generating resistance to the action of most of them, including inorganics, DDT, cyclodienes, organophosphates, carbamates, pyrethroids, juvenile hormone analogues, avermectins, neonicotinoids, and antimicrobial [22–24].

Though the use of pesticides has offered significant economic benefits by enhancing the production and yield of food and fibers and the prevention of vector-borne diseases, evidence suggests that their use has adversely affected the health of human populations and the environment [21]. Because of this problem, several researchers are focused their studies on the identification of new natural sources containing active metabolites that could be used on the control of pest [25, 26].

The development of essential oils (EOs) as plant protection products is especially suited to organic farming as well as to integrated pest management. They are natural in origin and biodegradable, have diverse physiological targets within insects, and, consequently, may delay the evolution of insect resistance [27]. EOs act as fumigants, pesticides, repellents, and antifeeds that could affect some biological parameters such as grown rate, biological cycle, and reproduction [26]. One of the most widely analyzed oils is neem (*Azadirachta indica*) which has a toxic effect over several pests and it is a potential alternative to the synthetic pesticide [28]. Neem oil active compounds are azadirachtin, salannin, nimbin, and their respective analogues; being the azadirachtin the most abundant compound [29]. Azadirachtin acts on the immature stages of the insects avoiding their molt or maturation from larva to pupa and generating mutations in the development of different essential parts for their survival, because it affects their ability to oviposit in mature stage and hatch during the larval stage [30]. Its effect is reinforced by the action from the rest of limonoides, such as salannin and nimbin, which have repellent and antifeeder effects over many insects [31]. Although the concentration of azadirachtin is sufficient and its location well established in the seed, its extraction presents some problems, because it is soluble in polar organic solvents, but slightly soluble in water, besides is photosensitive and thermolabile, which conditions its activity in the oil.

Among the methods used for the extraction of azadirachtin, it could be mentioned the cold extrusion in a mechanical press, maceration, and percolation with the use of organic solvents. Each of the proposed methods stimulates the extraction of azadirachtin but in different proportions. Various researchers have suggested that the high variability in the extraction of azadirachtin from neem depends on several factors as age of the tree, region of its production, stage of fruit development, availability of the internal portion of the seed, storage conditions of the seed, methods and solvents used for its extraction, and the particle size [32–35]. However, one aspect that has not been taken into account is the physical barrier exerted by the cell wall of the seed that directly affects the availability and extraction of azadirachtin and other acaricidal compounds. This problem has been overcome with great success in the plant extract industry through the application of cellulases or preparations with multiple enzymatic activities (cellulases, hemicellulases, and pectinases) [34, 36, 37].

The assisted extraction by cellulolytic enzymes has proven to be a viable and feasible tool to obtain bioactive metabolites from plants, due to its effect on lignocellulosic structures of its cell wall, which increase the yield of oils, pigments, flavorings, and aromas extracted in comparison with traditional extraction methods [38]. Due to the advantages of the use of cellulolytic enzymes for the production of bioactive metabolites from plants and the inherent need to develop sustainable and environmentally friendly alternatives that avoid the resistance phenomenon, the present study had the purpose of evaluating the use of food-grade enzyme preparation on the hydrolysis of the neem seed to obtain extracts with higher concentrations of azadirachtin, but without the use of solvents.

2. Assisted enzymatic extraction of neem

The study was divided into two stages: (1) determination of optimum activity conditions of four enzyme preparations and (2) evaluation of the azadirachtin release kinetics under optimal conditions of enzyme preparation activities. The enzyme preparations used in this study were Crystalzyme PML-MX, Cellulase 17600, Crystalzyme Cran, and Crystalzyme 100XL, and their optimum pH and temperature conditions were identified by using dehydrated and pulverized neem seed. Then, four volumes (1, 2, 3, and 4 mL) of each enzyme were tested to determine the optimum enzyme concentration to neem seed hydrolysis.

Evaluations of azadirachtin release kinetics were conducted at optimum pH and temperature for each enzyme preparation, and the maximum time required for the azadirachtin extraction was determined.

2.1. Material

Neem seeds were provided by a local producer from Jamapa, Veracruz during June, 2017. One kilogram of 110–120 day old neem seeds (green-yellow coloration) was collected. Harvested seeds were washed and cut in half to extract the cotyledon extraction, which was stored by in freezing at −20°C for 24 h before using.

2.2. Food-grade enzyme preparation cellulolytic activity

Each preparation enzymatic activity was measured by filter paper test [39], where the activity was defined as filter paper units per enzyme milliliter ($FPU{\cdot}mL^{-1}$). After $FPU{\cdot}mL^{-1}$ determination, the effect of the enzyme concentration on the hydrolysis of the neem seed cellulose structures and the azadirachtin release was evaluated. Neem seed hydrolysis was indirectly determined by the quantification of reducing sugar released during the enzymatic reaction. Total protein was estimated with [40] bovine serum albumin (BSA) as standard, and enzymatic activity (EA) for each enzymatic preparation was defined as changes in absorbance in 0.001 of reducing sugars mg protein^{-1} min^{-1}.

2.3. Optimum conditions of pH, temperature, and enzyme concentration

The optimal conditions of pH and temperature were established based on the enzymatic hydrolysis of the neem seed and the release of azadirachtin. To determine the optimum pH of the enzymatic preparations, 1 g of the dehydrated seed was homogenized with 19 mL of phosphate buffer (3.0, 3.5, 4.0, 4.5, 5.0, and 5.5) and was conditioned at 50°C for 5 min and after 1 mL of the corresponding enzyme preparation was added. The enzymatic reaction was carried out for 2 h and was stopped by immersion in ice water. Subsequently, 1 mL of the sample was taken for reducing sugar determination and 1 mL for azadirachtin quantification by HPLC [41]. Once the optimum pH of each enzyme preparation was identified, the effect of the temperature at 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70°C was evaluated. Additionally, one extract without enzyme added was prepared as control, and the concentrations of reducing sugars released were subtracted from the values obtained in each of the enzymatic treatments. Furthermore, enzyme:neem seed (dry base) ratio was evaluated under optimal conditions of pH and temperature, and for this, 2 g of neem seeds (wet base) were used and 0.5, 1.0, 2.0, and 4 mL of each of the enzyme preparations were tested.

2.4. Kinetics of azadirachtin release

To determine kinetics of azadirachtin release, 100 g of neem seed (wet base) was homogenized with phosphate buffer at the optimum pH previously identify (1:10, w v^{-1}) ratio for 3 min using an Ultra Turray homogenizer, T-25 basic (IKA®, Wilmington, NC). Five extracts were elaborated, one for each enzyme preparation and one without enzyme (control), and were incubated at optimum temperatures for each one. An aliquot was taken at 0, 2, 4, 6, 12, 18, and 24 h for the determination of azadirachtin. All samples were analyzed by triplicate.

2.5. Determination of azadirachtin

Azadirachtin quantification was carried out by the HPLC technique proposed by [41], using a binary HPLC system (Waters 1525) and a photodiode detector (Waters 2996). The analytical separation was carried out with a Nova-Pak C18 column of 4 μm (3.9 × 150 mm) SUM (Waters® Milford, MA). Neem samples were centrifuged and diluted with acetonitrile (1:1, v v^{-1}) before analyzing by HPLC. The samples were filtered through acrodiscs (Millipore) of 0.22 μm, and 20 μl was injected into the column. The flow rate was set at 1 mL min^{-1}, the mobile phase was acetonitrile: water (40:60, v v^{-1}), and azadirachtin was read at 217 nm in a retention time of 3.1 min.

2.6. Statistical analysis

The optimal conditions of pH, temperature, and the enzyme:substrate ratio were analyzed by means of analysis of variance (ANOVA) at a level of significance of $p < 0.05$, and Tukey's tests were used to evaluate the differences between means with the statistical program Minitab 17.3. The kinetics of release of reducing sugars and azadirachtin were analyzed by a first-order empirical equation $Y_i = Y_e (1−e^{-kt})$, in which the equilibrium concentrations (Y_e) and its release rate constant (*k*) were determined, and the results were analyzed by ANOVA ($p < 0.05$) to determine differences between treatments.

3. Results and discussion

3.1. Food-grade enzyme preparation activity

In order to achieve the optimum cellulolytic activity of the enzyme preparation, filter paper units (FPU·mL^{-1}) of each one were first determined. Crystalzyme PML-MX and Cellulase 17600 L showed the highest cellulolytic activities with 6.96 and 5.60 FPU·mL^{-1}, respectively. This result is probably due to the presence of cellulases in these enzymatic preparations, since the determination of the activity is carried out with filter paper, which is formed by cellulose [42], while the enzymatic preparations, Crystalzyme 100XL and Crystalzyme Cran, showed lower activities with 2.65 and 2.38 FPU mL^{-1}, respectively, and do not contain cellulases (**Table 1**). These results were used as reference to define the amount of enzyme needed to hydrolyze the neem seed cellulolytic structures and extract the highest concentration of azadirachtin.

3.2. pH and temperature determinations

Determination of the optimal reaction conditions was carried out at 50°C, taking into account that temperature is indicated by the enzyme preparation supplier as the optimum. However, in the evaluations, dehydrated neem seed was used instead of filter paper or crystalline cellulose. **Figure 1** shows the determinations of the optimum pH of each enzyme preparations, and the results indicate that all enzyme preparations perform the hydrolysis of the neem seed under very similar pH conditions regardless of the combinations of cellulolytic activity they contain. Optimal pH of the enzymatic preparations was 5.0 for Crystalzyme Cran and 4.5 for Crystalzyme PML-MX, Cellulase and Crystalzyme 100XL. Of the four enzyme preparations, Crystalzyme PML-MX exhibited the highest hydrolysis of the neem seed with 2.2114 (± 0.1879) mg reducing sugars mL^{-1}

Enzyme preparations	Cellulolytic activity	Activity (FPU mL^{-1})
Crystalzyme PML-MX	Pectinase, endoglucanase, exoglucanase, hemicellulase	6.96
Crystalzyme Cran	Pectinase	2.38
Crystalzyme 100XL	Pectinase and arabinase	2.65
Cellulase 17600	Endoglucanase, exoglucanase, β-glucosidase, pectinase and arabinoxylanase	5.6

Table 1. Enzymatic activities of the food-grade enzyme preparations.

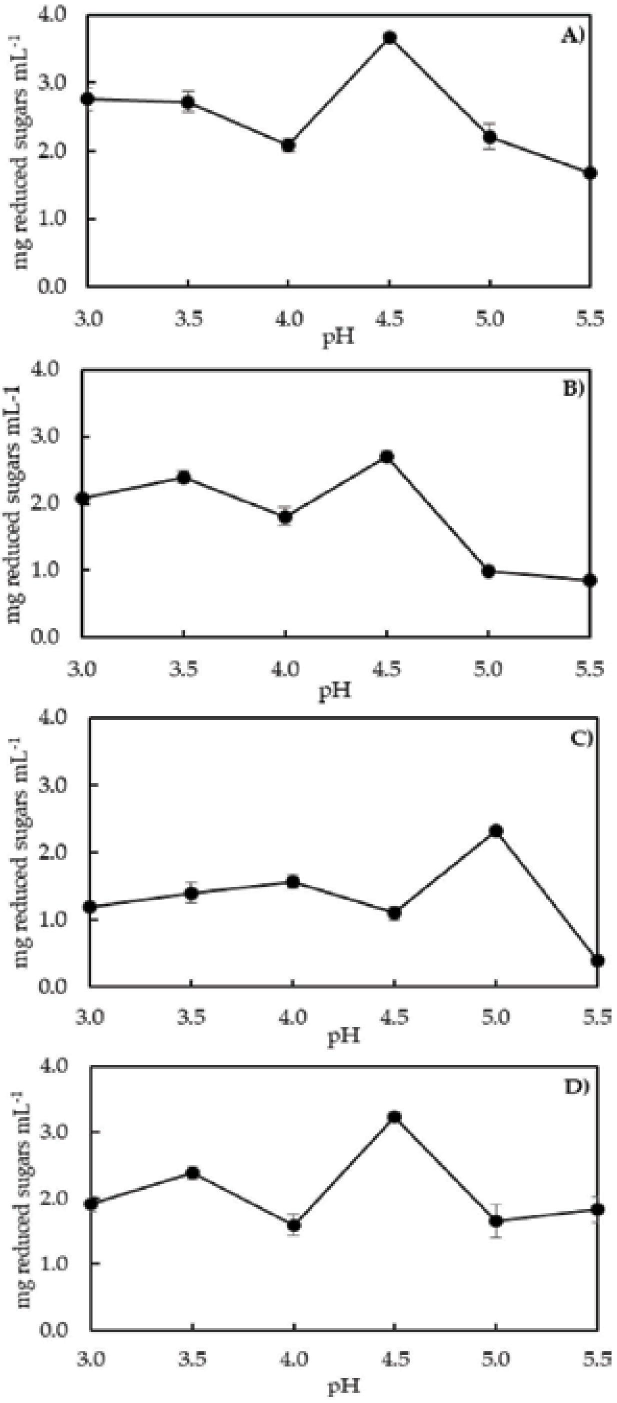

Figure 1. Optimum pH of food-grade enzyme preparations: (A) Crystalzyme PML-MX, (B) Cellulase 17600, (C) Crystalzyme Cran, and (D) Crystalzyme 100XL.

extract. A second reaction was carried out adjusting each hydrolysis to the optimum pH of each enzyme, and the effect of the temperature in the range of 25–70°C was evaluated (**Figure 2**).

Cellulase 17600L, Crystalzyme Cran, and Crystalzyme 100XL shown higher activity at 50°C, while to Crystalzyme PML-MX was at 45°C. These conditions are within the limit considered by [34] to maintain the neem extracts without the loss of azadirachtin. However, it is necessary to evaluate these conditions on the release of azadirachtin, since it is unknown whether the highest hydrolysis of the neem seed ensures maximum concentration of azadirachtin.

3.3. Enzyme concentration

Results of the enzyme concentration effect over hydrolysis of neem seeds could be observed in **Figure 3**. Reducing sugar release was used as indirect measure of neem seed hydrolysis.

Reactions catalyzed by Crystalzyme PML-MX and Cellulase 17600 L presented the highest hydrolysis of the neem seed, probably due to the presence of cellulases in its composition. A total of 1.8072 (± 0.0021), 2.0635 (± 0.0689), 2.0493 (± 0.0521), and 2.0742 (± 0.0283) g L^{-1} reducing sugars were released when 0.5 (3.48 FPU), 1 (6.96 FPU), 2 (13.92 FPU), and 4 mL (27.84 FPU) of Crystalzyme PML-MX were used. On another hand, 1.8034 (± 0.0387), 2.0809 (± 0.0023), 1.9921 (± 0.0456), and 1.9383 (± 0.0781) g L^{-1} reducing sugars were released when 0.5 (2.80 FPU), 1 (5.60 FPU), 2 (11.2 FPU), and 4 mL (22.4 FPU) of Cellulase 17600 were used. These results suggest that an increasing of enzyme concentration in the reaction not necessarily imply higher hydrolysis of the neem seed. Therefore, it is advisable to use the lowest concentration in which the same results were obtained for each enzyme preparation. Crystalzyme Cran and Crystalzyme 100XL showed less hydrolysis of the neem seed after 18 h. Crystalzyme Cran released 1.0022 (± 0.0576) g L^{-1} with the highest concentration of enzyme used (4 mL, 9.52 FPU); however, these results were not statistically different from those found when using 1 (2.38 FPU) and 2 mL (4.76 FPU), since the equilibrium concentration of reducing sugars was 0.9323 (± 0.0786) and 0.9859 (± 0.0341), respectively. On the other hand, the samples hydrolyzed with Crystalzyme 100XL had a maximum reducing sugar release when 4 mL of enzyme (21.2 FPU) was used.

In order to determine the optimum enzyme concentration to neem seed hydrolysis, equilibrium concentration and release rate of the reducing sugars were evaluated. The analysis of the results showed that reducing sugar release rate was not statistically different when 1, 2 or 4 mL for both Crystalzyme PML-MX and Cellulase 17600 L were used. When Crystalzyme Cran was evaluated, the higher rates of release of reducing sugars were obtained with 2 and 4 mL. In contrast, the highest reduced sugar release rate for Crystalzyme 100XL was presented when 1 mL (5.30 FPU) of this enzyme was added. Therefore, based on the analysis of results, it was considered that the units of activity necessary to hydrolyze the neem seed are 6.96, 5.60, 4.76, and 5.3 FPU mL^{-1} for Crystalzyme PML-MX, Cellulase 17600 L, Crystalzyme Cran, and Crystalzyme 100XL, respectively.

After optimum conditions of pH, temperature and enzyme concentration were determined, and the kinetics of azadirachtin released was carried out but using fresh seed instead of dehydrated seed to minimize the risk of losses due to the dehydration process.

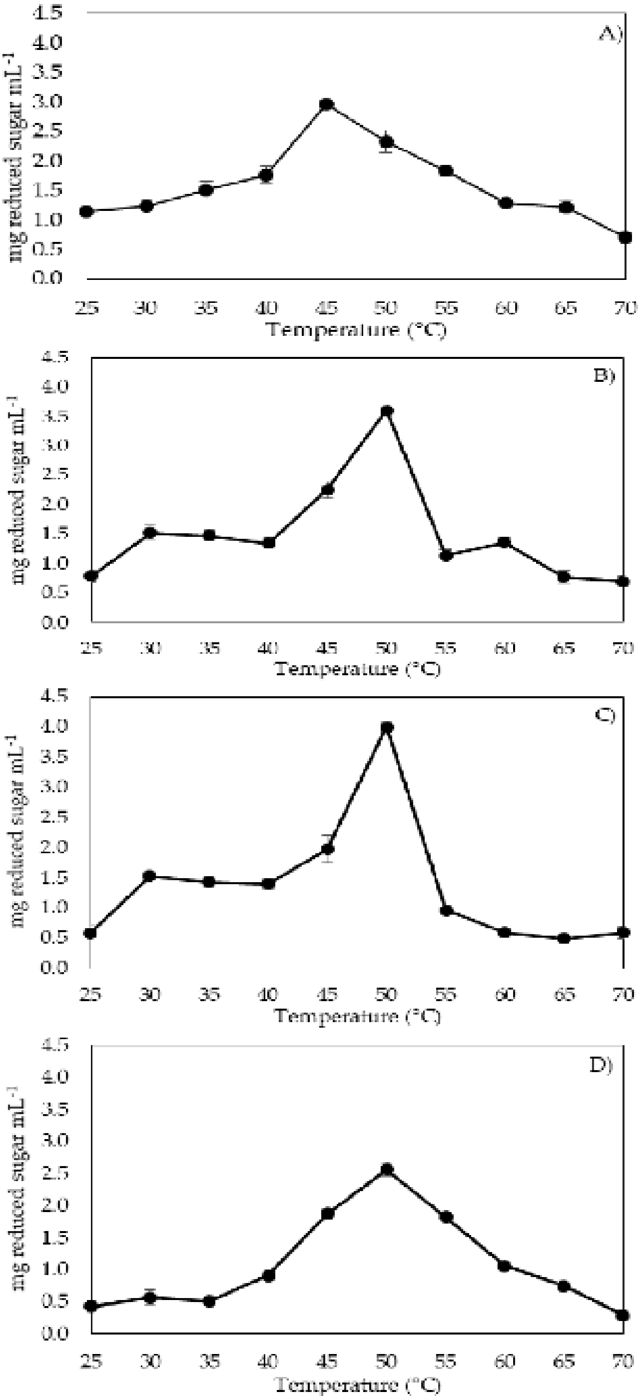

Figure 2. Optimum temperature of food-grade enzyme preparations: (A) Crystalzyme PML-MX, (B) Cellulase 17600, (C) Crystalzyme Cran, and (D) Crystalzyme 100XL.

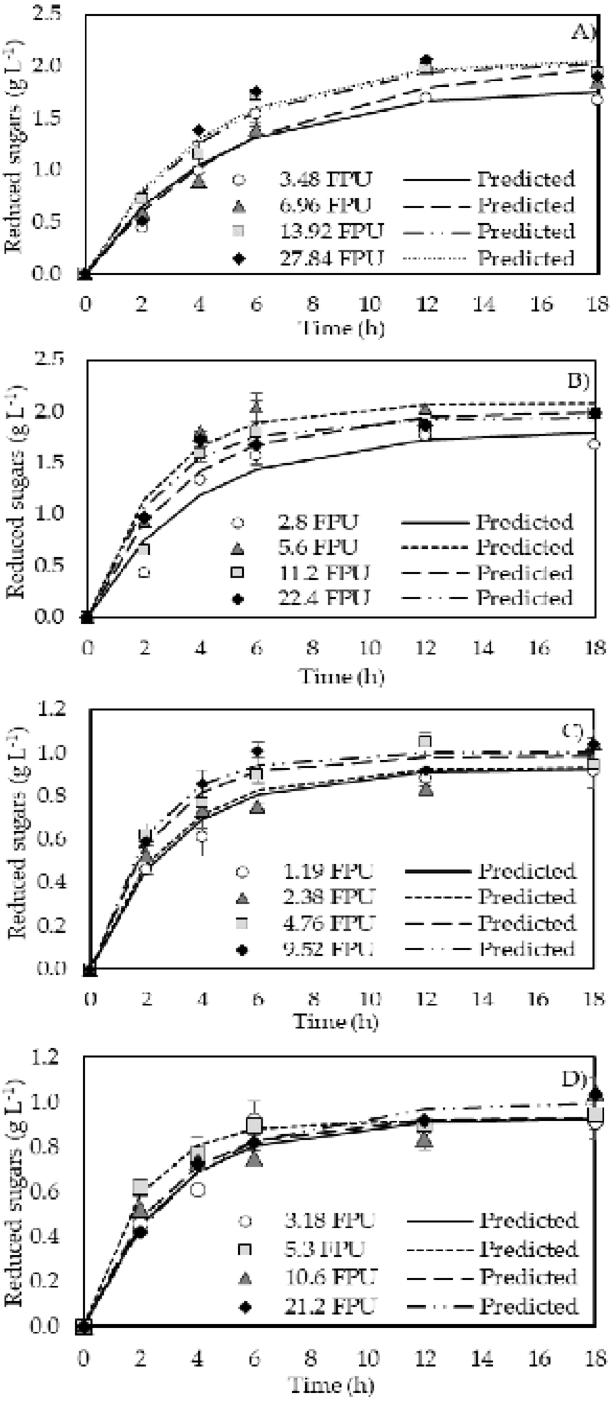

Figure 3. Optimum filter paper unit (FPU) of the food-grade enzyme preparations for hydrolysis of neem seeds. (A) Crystalzyme PML-MX, (B) Cellulase 17600, (C) Crystalzyme Cran, and (D) Crystalzyme 100XL.

3.4. Azadirachtin release kinetics

Evaluation of the azadirachtin release kinetics was conducted with 100 g of neem seed homogenized and adjusted at 1:10 (w v^{-1}) ratio with phosphate buffer. Enzymatic hydrolysis was conducted under optimal conditions of each enzyme preparation, and azadirachtin were quantified at 0, 2, 4, 6, 12, 18, and 24 h by the HPLC technique, and the results were presented on base of the quantity of neem seed (dry base) used for the neem seed extract elaboration (**Figure 4**). The highest azadirachtin concentrations obtained in this study were 2.55 g kg^{-1} (2550 ppm) and 2.35 g kg^{-1} (2350 ppm) neem seed when Cellulase 17600 L and Crystalzyme PML-MX enzyme preparations were used, respectively. Both of this enzyme preparation include cellulases and pectinases and were statistically different ($p < 0.05$) from the enzymatic preparations, Crystalzyme Cran (pectinases) and Crystalzyme 100XL (pectinases and arabinases).

Azadirachtin concentrations found in this study were higher than those reported by other authors when conventional methods such as extrusion, extraction with solvents (hexane, methanol), and aqueous extraction which reported concentrations of 1080, 565, 400, 150 ppm, respectively [33] and were similar to the obtaining by cold methanol extrusion [32]. However, the concentration obtained is lower than those reported with nonconventional technologies such as extraction with pressurized solvents, which reported concentrations of up to 9510 ppm of azadirachtin [34].

One of the advantages of the use of organic solvents in the extraction process is to improve the solubility of nonpolar components which increase their extraction from vegetable matrices. However, in the present study, only phosphate buffer was used, which could explain the differences of azadirachtin extraction with the pressurized solvent method [34]. Also, in [43], it was reported that in the tropical regions, there are lower concentrations of azadirachtin after extraction processes due to high temperatures, moisture, and storage conditions that could encourage the azadirachtin degradation.

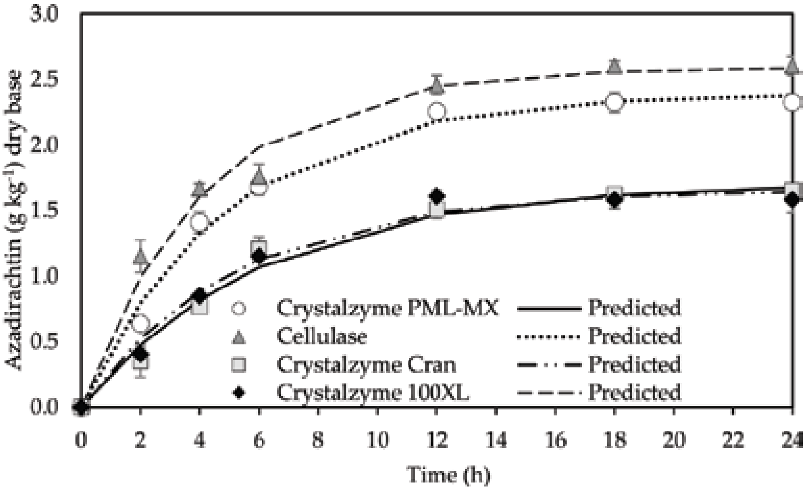

Figure 4. Azadirachtin release kinetics from neem seeds extracted with food-grade enzyme preparations.

Although the highest concentrations of azadirachtin were obtained with the enzymatic preparations, Cellulase 17600L and Crystalzyme PML-MX, also Crystalzyme Cran and Crystalzyme 100XL could be used to elaborate neem extracts because of their azadirachtin concentrations of 1540 and 1690 ppm, respectively, are similar to those contained in commercial acaricidal products elaborated based on neem, but with the use of solvents [32]. This result indicates an advantage and a possible solution to the indiscriminate use of synthetic pesticides and the organic solvents employed in several extractions of active biomolecules. On the other hand, after 18 h of enzymatic hydrolysis, there are no changes on azadirachtin release, which means it is time required to carry out the obtaining of neem seed extracts. In addition, the conditions identified in this study as necessary to neem seed hydrolysis are not extreme or aggressive and could be easily scalable.

4. Conclusions

The present study had the objective of generating an alternative solution to the multiple problems generated by the indiscriminate use of synthetic pesticides. The use of enzymes in the production of metabolites with biological interest has been widely studied in the last decade, due to their specificity and their effect on the cellulose substrate hydrolysis, minimizing the times of obtaining and the necessary costs for their implementation. In addition, its application allows reducing the use of solvents, normally employed in the extraction of neem oil. Besides, the conditions required for neem seed hydrolysis under optimal activity of the enzyme preparations can be easily standardized and scaled for its industrial production. In addition, it was found no affectations on azadirachtin concentration when temperatures of 45 and 50°C were used during enzymatic hydrolysis coinciding with that reported in [34]. However, more studies are needed to determine the stability of azadirachtin under different temperatures and pH conditions during storage.

Although the enzyme-assisted extraction allows the obtaining of neem extracts with higher azadirachtin concentrations than those obtained with conventional methods such as extrusion, cold extrusion, water maceration, and percolation with hexane [32, 33] and in similar concentrations to those obtained by extrusion with cold methanol [32], the amount of neem seeds used in this method was lower, and therefore, the yield was higher than obtained with all these methods. In next studies, components identified as repellents and/or acaricides such as salannin and nimbin should be analyzed. In addition, it is necessary to carry on short-term and long-term *in vitro* and *in vivo* analysis of enzymatic neem extracts on pest with public health impact, defining the vehicle of application and the lethal concentrations for its implementation.

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Conflict of interest

All authors who participate in the elaboration of this manuscript have no conflict of interest to declare.

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