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# Pesticides in the Environment: Impacts and Their Biodegradation as a Strategy for Residues Treatment

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## 1. Introduction

A vast number of pollutants and waste materials including heavy metals are disposed into the environment per annum. Approximately  $6 \times 10^6$  chemical compounds have been synthesized, with 1,000 new chemicals being synthesized annually. Almost 60,000 to 95,000 chemicals are in commercial use. According to Third World Network reports, more than one billion pounds (450 million kilograms) of toxins are released globally in air and water. The contaminants causing ecological problems leading to imbalance in nature is of global concern. The environmentalists around the world are trying to overcome this by several means. However, although they are raising their voices at international platforms regarding the depletion of natural resources; little attention is given to their words and many substances are still used without caring of the adverse consequences (Shukla et al., 2010).

Among these chemicals we can find pesticides, which are defined as any substance or mixture of substances which are used to control destructive pests such as insects, plant disease organisms and weeds, including many other living organisms such as nematodes, arthropods other than insects, and vertebrates that endanger our food supply, health, or comfort. In particular, the term pesticide refers to chemical substances that alter biological processes of living organisms deemed to be pests, whether these are insects, mould or fungi, weeds or noxious plants. Pesticides are widely used in most areas of crop production to minimize infestations by pests and thus protect crops from potential yield losses and reduction of product quality (Damalas, 2009). These pests potentially cause damage or interfere in any other way in the production, elaboration, storage, transport, or commercialization of food, agricultural products and wood products or animal food. Pesticides may be given to animals to prevent insects, arachnids or other plagues in or over their bodies (FAO, 2002).

Certainly, pesticides have improved longevity and quality of life, chiefly in the area of public health. Insect control programs have saved millions of lives by combating diseases such as malaria, yellow fever and typhus. In addition, the use of pesticides constitutes an important aspect of modern agriculture, as they are absolutely necessary for economical pest management (Gouma, 2009). The most promising opportunity for maximizing benefits and minimizing risks is to invest time, money, and effort into developing a diverse toolbox of

pest control strategies that include safe products and practices that integrate chemical approaches into an overall and ecologically based framework which will optimize sustainable production, environmental quality, and human health (Damalas, 2009).

However, the application of pesticides may cause adverse effects among the different forms of life and among the ecosystems; this will depend on the sensibility grade of the organisms and the pesticide (CICOPLAFEST, 2004). Approximately 90% of agricultural pesticide application never reaches its target organisms but is, instead, dispersed through the air, soil, and water. As a result, they are routinely detected in air, surface and ground water, sediment, soil, vegetable, and to some extent in foods. In addition, many soil-applied pesticides are also intentionally introduced into the soil environment for the control of soil borne pests and pathogens, which results in the accumulation of their residues and metabolites in soil at unacceptably high levels (Gamón et al., 2003; Shalaby & Abdou, 2010).

The inadequate application practice is one of the most important ways of pollution, which has a profound impact not only on the soils of the areas in which they are applied. Pesticides are washed into aquatic ecosystems by water runoff and soil erosion. Pesticides also can drift during application and contaminate aquatic systems. Some soluble pesticides are easily leached into streams and lakes. Wild birds and mammals are damaged and destroyed by pesticides and these animals make excellent "indicator species". Deleterious effects on wildlife include death from the direct exposure to pesticides or secondary poisonings from consuming contaminated food; reduced survival, growth, and reproductive rates from exposure to sub-lethal dosages; and habitat reduction through the elimination of food resources and refuges. Pesticides easily find their way into soils, where they may be toxic to arthropods, earthworms, fungi, bacteria, and protozoa. Small organisms are vital to ecosystems because they dominate both the structure and function of ecosystems. Like pest populations, beneficial natural enemies and biodiversity (predators and parasites) are adversely affected by pesticides. Fungicides also can contribute to pest outbreaks when they reduce fungal pathogens that are naturally parasitic on many insects. When outbreaks of secondary pests occur because their natural enemies are destroyed by pesticides, additional and sometimes more expensive pesticide treatments have to be made in efforts to sustain crop yields. This raises the overall costs and contributes to pesticide-related problems. In addition to destroying natural enemy populations, the extensive use of pesticides has often resulted in the development and evolution of pesticide resistance in insect pests, plant pathogens and weeds (Pimentel, 2005; Aktar et al., 2009).

Besides, pesticide application generates social conflicts because of the elevated number of workers who are intoxicated by these products, with a high mortality rate, as well as for the suspicion of adverse effects on the health of surrounding communities, flora and fauna (Lichtinger *et al.* 2001). These toxic compounds have been implicated in various disorders and diseases including cancer, adverse reproductive outcomes, peripheral neuropathies, neurobehavioral disorders, impaired immune functions and allergic sensitization reactions, particularly of the skin, cumulative inhibition of cholinesterase activity because of long-term low doses of exposure (Al-Qurainy & Abdel-Megeed, 2009).

The metabolic fate of pesticides is dependent on abiotic environmental conditions (temperature, moisture, soil pH, etc.), microbial community or plant species (or both), pesticides characteristics (hydrophilicity, level of solubility) and biological and chemical reactions. Abiotic degradation is due to chemical and physical transformations of the pesticide by processes such as photolysis, hydrolysis, oxidation, reduction and rearrangements. Furthermore, pesticides may be biologically unavailable because of

compartmentalization, which occurs as a result of pesticide adsorption to soil and soil colloids without altering the chemical structure of the original molecule. However, enzymatic transformation, which is mainly the result of biotic processes mediated by plants and microorganisms, is by far the major route of detoxification (Van Eerd et al., 2003).

Another important concern is that the millions of tons of pesticides applied annually in a worldwide level generate liquid and solid wastes. Besides, containers many times are placed directly and without control on the soil and water mainly, polluting and affecting the food chains (Ortiz-Hernández et al., 1997). Among the main consequences derived from the soil pollution, we find the loss of fertility, which directly or indirectly allow the survival of the flora and fauna, given the tight interrelationships among the different elements, which constitute the ecosystems.

On the other hand, there are more than half a million tons of obsolete, unused, forbidden or outdated pesticides, in several developing and transitional countries, which endanger the environment and health of millions of people. In the absence of a clear obsolete pesticides management strategy, over the years, significant amounts of obsolete pesticides have been stockpiled in developing countries (Dasgupta et al., 2010).

An obsolete pesticide may be recognized as one that is undesirable or impossible to use and has to be eliminated, these include (Martinez, 2004; Karstensen et al., 2006; Shah & Devkota, 2009; Dasgupta et al., 2010):

- Technical pesticides and formulations passing the expiration date (generally two years after their manufacture).
- Pesticides whose use has been forbidden or strictly restricted.
- Damaged products:
  - Those who suffered physical or chemical changes which make them phytotoxic for the crops, or with non acceptable dangerousness for human and environment health.
  - Those who suffered a loss of biological efficiency.
  - Those who present changes in their physical properties which make them incompatible with the habitual equipments of application.
- Pesticides which are undesired by their owners, even if they are in good conditions for their use.
- Products without identification.
- Products which are polluted with other substances.

It is also included:

- a. Pesticide wastes generated on fire and other accidents.
- b. Materials that are strongly polluted with pesticides.
- c. Wastes that are generated by the fabrication or formulation of pesticides.

Because of their characteristics, obsolete pesticides are a dangerous waste, that is why they should be managed as such. Obsolete pesticides have accumulated in almost every developing country or economy in transition over the past several decades (Dasgupta et al., 2010). It is estimated that in Africa and Middle East there are more than 100,000 tons of these products, in Asia almost 200,000 and a similar quantity in East Europe and the old Soviet Union. Nowadays the FAO is elaborating the inventories of Latin America (Farrera, 2004; Karstensen et al., 2006; Ortiz-Hernández & Sánchez-Salinas, 2010).

In México, there is knowledge of the existence of obsolete pesticide products, both liquid and solid. A total of 551 records of obsolete pesticide products have been registered,

distributed in 29 of 33 states of Mexico, achieving a total of 26,725.02 liters, 147,274 kg and 500 m<sup>3</sup> of highly polluted soils. In addition there are 28 reports of pesticide-contaminated sites in 15 states of the Mexican Republic (Giner de los Ríos, 2007). Besides, some data indicate that the total of empty pesticide containers can be about 7,000 tons annually (Albert, 2005).

Many works point to health or environmental problems from accidental or deliberate exposure to pesticides, particularly pesticides with high mammalian toxicity or those that persist in the environment. These risks should not be ignored, and efforts must be made to minimize them through rigorous regulation and proper training for users, because we should not overlook the positive impacts of pesticide use. When pesticides are used rationally and carefully, in conjunction with other technologies in integrated pest management systems, it is more likely that their use will be justifiable (Cooper & Dobson 2007).

## **2. The need for pesticides treatment and final disposition: biotechnological strategies**

The damages caused to the environment and health, such as the existence of obsolete pesticides, make necessary the development of technologies that guarantee their elimination in a safe, efficient and economic way. Among the existent technologies there are those that apply physical treatments, such as adsorption and percolator filters; chemical treatments such as the advanced oxidation or inverse osmosis, and incineration, a treatment option usually not available in developing countries (Karstensen et al., 2006). However, a treatment that promises to be efficient, economic and safe is the biological treatment, because several reactions catalyzed by enzymes of specific microorganisms take place. This kind of treatment has been approached from a biotechnological point of view in order to be able to have a methodology that is safer and more economic than the conventional treatments, as well as avoiding additional damages to the environment. Biological processes have been used to give treatment to wastes and polluted sites with pesticides (Araya & Lakhi, 2004). Among them, the microbial metabolism is the primary force of transformation or degradation. In many cases it has been reported that the microorganisms are very important in the degradation of xenobiotic compounds. Biological treatment can be applied to compounds whose chemical structure in the nature is infrequently or inexistent because they are synthesized artificially (Ortiz-Hernández, 2002). The importance of the microorganisms lays on their great diversity and metabolic plasticity, which allows them to use diverse ecological niches. Many microorganisms may live in a large diversity of media because of their remarkable capacity of mutation and adaptation; besides, they seem to have a great potential to acquire capacities of degradation when they are exposed to xenobiotics. Additionally, isolated microorganisms with the capacity to degrade xenobiotic compounds have the potential to be used for the bioremediation of other compounds that do not have any known microbial system for their degradation (Singh & Walker, 2006). Biodegradation of these pesticides provides a cheap and efficient solution for their final disposal or for treatment of agricultural soils, contaminated water or polluted ecosystems. In 1973, the first bacteria with the capability of degrading organophosphorus compounds were described (Sethunathan, 1973; Sethunathan & Yoshida, 1973, Siddaramappa et al., 1973). Since then, a number of different genera have been identified, and the enzymes involved in pesticide degradation have been studied.

### 3. Obtaining isolates that degrade pesticides

The role of microorganisms in the dissipation of pesticides, especially in the soil, has long been recognized. Relative to the extended evolutionary period of microorganisms in nature, agriculture has only been around for about 10,000 years, and the introduction of organic pesticides is only a half-century old. Therefore on an evolutionary scale, the time for microbial adaptation for degrading the influx of new xenobiotic compounds is exceptionally short, and it is an ongoing process. There is much evidence from observational and molecular research that indicate microbial adaptation for the mineralization of pesticide has occurred since their first introduction into agriculture in the mid-1950's.

The use of pesticides over time, has resulted in several microorganisms which are able to degrade xenobiotic organic compounds, including pesticides, using different strategies and enzymatic pathways. The first organophosphate pesticide-degrading bacterial strain was isolated from a paddy field in the Philippines in 1973. Since then, several phylogenetically distinct bacteria that can degrade pesticides by co-metabolism, or use them as a source of carbon, phosphorus or nitrogen, have been isolated from different parts of the world (Table 1).

Because pesticides are mainly applied to agricultural crops, soil is the medium that mostly gets these chemicals. Once in the soil, pesticides follow different ways such as degradation, volatilization, sorption, or surface transport to other places. Studies have shown that biodegradation is a process that majority occurs in pesticide dissipation, which is due to the adaptation of microorganisms after having been in contact with soils with extensive exposure to pesticides. Some findings indicate that in most cases the half-lives of pesticides in soils with a history of pesticide application are considerably shorter than in those in which no application has been performed. Besides, the pesticides that were considered non-biodegradable become biodegradable after a number of years. Therefore, the soil is a major source to isolate microorganisms that degrade pesticides (Table 1), which is an opportunity for use in the treatment of waste or obsolete pesticides.

Other sources of microorganisms with the ability to degrade pesticides are: pesticide industry's effluent- sediment, sewage sludge, activated sludge, wastewater, natural waters, sediments, areas surrounding the manufacture of pesticides, and even some live organisms. In general, microorganisms that have been identified as pesticide degraders have been isolated from a wide variety of sites contaminated with some kind of pesticide.

For the isolation of soil microorganisms, the most used procedure is as follows. Microbial population in a sample of soil is cultivated in constant agitation in flasks of different capacities with mineral salts medium using a particular pesticide (or mixture of pesticides) as the only source of carbon. This procedure is repeated several times, increasing the concentration of pesticides to ensure the adaptation of microorganisms to the conditions of the culture in the laboratory as well as the growth of those that used the pesticide as their only source of carbon. After the adaptation time, several bacterial consortiums are obtained. These consortiums do not need an additional source of carbon, since the pesticide they were adapted to is enough. At present, in different laboratories around the world, there are collections of microorganisms characterized by their identification, growth and degradation of pesticides. The isolation and characterization of microorganisms that are able to degrade pesticides give the possibility to count with new tools to restore polluted environments or to treat wastes before the final disposition.

Microorganism	Pesticide	Place of isolation	Reference
Bacteria			
<i>Ochrobactrum</i> sp.	Methyl parathion	Soil	Qiu et al., 2006
<i>Arthrobacter</i> sp.	Endosulfan	Soil	Weir et al., 2006
<i>Sphingomonas</i> spp.	Isoproturon	Soil	Bending & Rodríguez, 2007
<i>Burkholderia</i> sp.	Fenitrothion	Soil	Hong et al., 2007
<i>Sphingomonas</i> sp.	Chlorpyrifos	Wastewater	Li et al., 2007
<i>Enterobacter</i> spp.	Chlorpyrifos	Soil	Singh et al., 2004
<i>Acinetobacter radioresistens</i>	Methyl parathion		Liu et al., 2007
<i>Ochrobactrum</i> sp., <i>Castellaniella</i> sp., <i>Variovorax</i> sp., <i>Pseudomonas</i> sp., <i>Psychrobacter</i> sp.	Igepal CO-210 Igepal CO-520	Sewage sludge	DiGioia et al., 2008
<i>Pseudomonas frederiksbergensis</i>	Dimetoate, Malathion	Soil	Al-Qurainy & Abdel-Megeed, 2009
<i>Bacillus pumilus</i>	Chlorpyrifos	Soil	Anwar et al., 2009
<i>Bacillus</i> sp.	Mesotrione	Soil	Battison et al., 2009
<i>Serratia liquefaciens</i> , <i>Serratia marcescens</i> , <i>Pseudomonas</i> sp.	Diazinon	Soil	Cycón et al., 2009
<i>Enterobacter aerogenes</i>	Bifenthrin Fenpropathrin Cypermethrin	Sewage sludge	Liao et al., 2009
<i>Pseudomonas putida</i> , <i>Burkholderia gladioli</i> .	Prophenofos	Soil	Malghani et al., 2009
<i>Stenotrophomonas</i> sp.	DDT	Soil	Mwangi et al., 2010
<i>Providencia stuartii</i>	Chlorpyrifos	Soil	Rani et al., 2009
<i>Pseudomonas putida</i>	Propiconazole	Tea rhizosphere	Sarkar et al., 2009
<i>Micrococcus</i> sp.	Diuron	Diuron storage	Sharma et al., 2010
<i>Sphingobium</i> sp.	Methyl parathion Fenpropathrin	Sewage sludge	Yuanfan et al., 2010

Table 1. Some organisms isolated that degrade different pesticides.

Fungus			
<i>Aspergillus niger</i>	Endosulfan	Soil	Bhalerao & Puranik, 2007
<i>Ganoderma australe</i>	Lindane	<i>Pinus pinea</i> stump	Rigas et al., 2007
<i>Trichosporon</i> sp.	Chlorpyrifos	Sewage sludge	Xu et al., 2007
<i>Verticillium</i> sp. DSP	Chlorpyrifos	Soil	Fang et al., 2008
<i>T. versicolor</i> (R26)	Atrazine	Soil	Bastos & Magan, 2009
<i>Aspergillus sydowii</i> , <i>Bionectria</i> sp., <i>Penicillium miczynskii</i> , <i>Trichoderma</i> sp.	DDD	Marine sponge	Ortega et al. 2010
Algae			
<i>Chlorophyceae</i> sp., <i>Scenedesmus</i> spp., <i>Chlamydomonas</i> sp., <i>Stichococcus</i> sp., <i>Chlorella</i> sp. Cyanobacteria <i>Nostoc</i> spp.	Fenamiphos	Soil	Cáceres et al., 2008
<i>Anabaena</i> sp.	Fenamiphos	Water	Cáceres et al., 2008
Yeast			
<i>Lipomyces kononenkoae</i>	Picloram	Soil	Sadowsky et al., 2009

Table 1. (Continued)

#### 4. Microbial degradation of pesticides: genes and enzymes

Microbial metabolism has proved to be very versatile and diverse. This characteristic has allowed many different bacterial and fungal genera to evolve activities capable of xenobiotic degradation and offers an important source of alternatives for bioremediation.

Bioremediation of pesticides provides a cheap and efficient solution for their final disposal or for treatment of agricultural soils, contaminated water or polluted ecosystems. Microbial degradation has advantages because a large variety of compounds can be degraded completely under mild conditions compared with degradation using physical and chemical means.

Most of the research regarding pesticide degradation by microorganisms has been performed mainly with bacteria; a few studies have focused on fungi, actinomycetes, cyanobacteria, etc. This obeys mainly to the fact that bacteria are easy to culture in simple media and grow faster than other microbes; besides, bacteria are more susceptible to genetic modifications, which give them an extra potential to increase their degradation capabilities.

Bacterial genetics and molecular biology tools have contributed widely to the understanding of the degradation processes and to the isolation and characterization of genes involved in pesticide degradation. It is important to note, however, that in nature pesticide mineralization is accomplished by microbial communities rather than isolated species and that in many instances co-metabolic pathways are used.

Since the chemical structure of pesticides is variable, individual reactions of degradation–detoxification pathways are versatile and include oxidation, reduction, hydrolysis, and conjugation. These reactions are achieved through a number of different enzymes such as dehydrogenases (Bourquin, 1977; Singh & Singh, 2005), dioxigenases (Nadeau et al., 1994; Van Eerd et al., 2003), cytochrome p450 (Castro et al., 1985; Jauregui et al., 2003), ligninases (Pizzul et al., 2009) and, in the case of organohalogenate compounds, dehalogenases (Franken et al., 1991; Sharma et al., 2006). Conjugation with glutathione is commonly used as a detoxification mechanism, especially in plants and insects, although this mechanism has also been reported in bacteria (Vuilleumier, 2001; Wei et al., 2001; Chaudhry et al., 2002).

Metabolism of pesticides may involve a three-phase process. In Phase I metabolism, the initial properties of a parent compound are transformed through oxidation, reduction, or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent. The second phase involves conjugation of a pesticide or pesticide metabolite to a sugar or amino acid, which increases the water solubility and reduces toxicity compared with the parent pesticide. The third phase involves conversion of Phase II metabolites into secondary conjugates, which are also non-toxic. In these processes fungi and bacteria are involved producing intracellular or extra cellular enzymes including hydrolytic enzymes, peroxidases, oxygenases, etc (Van Eerd et al., 2003).

Here we will revise some aspects of microbial degradation of the most important groups of pesticides.

#### 4.1 Organochlorines

Although organochlorine pesticides are less used every day and many countries have banned their application, in some countries they still represent a problem of disposal, since this group of pesticides is the most persistent. There are two major pathways through which microorganisms degrade organochlorine compounds: reductive dechlorination, a process that takes place under anaerobic conditions, and dehydrochlorination, occurring in the presence of oxygen. Several bacterial genera have been proven to undertake these reactions including *Klebsiella* (Kwon et al., 2005), *Alcaligenes* (Don & Pemberton, 1981) *Staphylococcus*, (Sonkong et al., 2008), and *Pseudomonas* (Barragan-Huerta et al., 2007).

Several reports have documented the capability of different genera of fungi to degrade organochlorines. Among these, basidiomycetes seem to be more resistant to these compounds (Gomes Machado et al., 2005; Rigas et al., 2005). Recently a strain of *Trichoderma harzianum* has also been shown to degrade organochlorines through an oxidative system (Katayama & Matsumura, 2009).

#### 4.2 Organophosphates

This group of pesticides have been widely used as pesticides or chemical warfare agents because of their high toxicity towards insects, mammals and other animals. Their mechanism of action involves the irreversible inhibition of acetylcholine esterase, a key enzyme of the central nervous system, thus affecting non-target organisms as well (Singh & Walker 2006). Despite this, they are still used worldwide in large quantities as pesticides. Other organophosphorus compounds, such as nerve gases are still stored in important amounts and have to be destroyed. The basic structure of organophosphorus pesticides consists of ester or thiol derivatives of phosphoric, phosphonic or phosphoramidic acids.

The main degradation pathway starts with the hydrolysis of the P-O alkyl or P-O aryl bonds, which diminishes as much as a 100 times the toxicity of these compounds. Bacterial

enzymes have been found to achieve such detoxifying reactions (Singh et al., 1999; Yañez-Ocampo et al., 2009; Yañez-Ocampo 2009). This reaction is performed by esterases or phosphotriesterases that have been described for a number of different genera of bacteria and fungi. Among these, several different genes have been described (Singh & Walker, 2006).

A large group of bacterial genera has been reported to degrade organophosphates compounds. The studied and reported enzymes are related to the phosphotriesterase, which is capable of hydrolyzing organophosphate pesticides in the central atom of pesticides' phosphorus. Hydrolysis is fundamental for the complete degradation of the molecule. Phosphotriesterase activity is the first and most important step in detoxification. Several genes encoding for different phosphotriesterase activities have been described from a number of organisms (including fungi), the most studied being the *opd* and *opaA* genes that code for organophosphorus hydrolase (OPH) and organophosphorus acid anhydrolase (OPAA), respectively (Singh & Walker 2006). *opd* genes have been described in *Flavobacterium* and *Pseudomonas* species and are plasmid borne (Serdar et al., 1982; Somara & Siddavattam, 1995), while a similar gene, *opdA*, is present in *Agrobacterium radiobacter*'s chromosome (Horne et al., 2002). *opaA* genes were found in *Alteromonas* species and are chromosomally located (Cheng et al., 1996; Cheng et al., 1997). Another gene coding for organophosphates hydrolase, *mpd*, described originally in *Plesiomonas* sp. (Zhongli et al., 2001), has also been found in other genera like *Achromobacter*, *Pseudaminobacter*, *Ochrobactrum* and *Brucella* and is located in the chromosome (Zhang et al., 2005). Other important bacterial genera able to degrade organophosphates include *Burkholderia* (Zhang, et al., 2006) and *Hyphomicrobium* (Wang et al., 2010).

Some fungal species have been reported to degrade organophosphates. Amitai et al., (1998), reported that laccase, a broad spectrum phenol oxidase from the white rot fungus *Pleurotus ostreatus*, could hydrolyze P-S bonds, which are resistant to bacterial phosphotriesterase hydrolysis; other organophosphate compounds such as nerve gases could also be hydrolyzed by this enzyme (Amitai et al., 1998). It remains to be explored if laccases from other species can also hydrolyze these compounds, since their main mechanism is an oxidative one.

Ascomycetes such as *Aspergillus* and *Penicillium* have also been shown to produce organophosphate degrading enzymes other than laccases (Liu et al., 2001; Liu et al., 2004). The *Penicillium* enzyme (P-OPH) is more versatile since it can hydrolyze P-S and P-O linkages whereas the *Aspergillus* A-OPH only splits P-S bonds and the bacterial enzymes can only hydrolyze P-O bonds.

### 4.3 Carbamates

Carbamates are used to control insects and nematodes in soils. Their toxicity to mammals is very high although they are not as persistent as organochlorines. A number of bacterial genera have been identified as carbamate degraders (Parekh et al., 1995). Degradation of the pesticide occurs mainly through the hydrolysis of the methylcarbamate linkage by an enzyme called carbofuran hydrolase, codified by the *mcd* gene, which was located on a plasmid first described in *Achromobacter* sp. (Tomasek & Karns, 1989). Further studies showed that a wide variety of bacteria could degrade carbamates using carbofuran hydrolase. Among other genera *Pseudomonas*, *Mesorhizobium*, *Ralstonia*, *Rhodococcus*, *Ochrobactrum*, and *Bacillus* are the most notorious (Desaint et al., 2000).

Fungal degradation of carbamates has also been reported. Of special interest is the report a novel hydrolase from *Aspergillus niger* capable of hydrolysing several N-methylcarbamate insecticides (Qing et al., 2006).

#### 4.4 Pyrethroids

Pyrethroids insecticides are a class of lipophilic esters, with an alcohol and an acid moiety. Although less toxic and persistent than other groups of insecticides, they can still represent a problem. Pyrethroids display high affinity to Na<sup>+</sup>-channels and its binding to these channels causes a prolonged channel opening that may result in a complete depolarisation of the cell membrane thus blocking neuronal activity. There are two main routes of degradation, photo- and biodegradation, which are often superimposed. Pyrethroids developed for use in agriculture are much more photostable than the natural pyrethrins but they are still sensitive to sunlight, which provokes isomerisation or ester cleavage. The basic pathway of pyrethroid degradation by microbes proceeds through the hydrolysis of the main ester linkage. This reaction can be carried out by carboxyl esterases or phosphotriesterases.

Bacterial degradation of pyrethroids has been documented. A report by Grant et al. (2002) describes the ability to degrade synthetic pyrethroids by *Serratia* and *Pseudomonas* isolates. *Bacillus*, *Achromobacter* and *Pseudomonas fluorescens* have also been studied in regard to pyrethroid degradation (Maloney et al., 1988). Another interesting report shows the degradation of allethrin, a recalcitrant pyrethroid used in mosquito mats by an *Acidomonas* sp. strain (Paingankar et al., 2005).

Fungal degradation of pyrethroids seems promising. Ascomycetes (*Trichoderma*, *Aspergillus*) as well as basidiomycetes (*Phanerochaete*) have been reported to degrade pyrethroids through the cleavage of ester bond (Saikia and Gopal, 2004). A novel pyrethroid hydrolase from cell extracts of *Aspergillus niger* has also been characterized (Liang et al., 2005).

### 5. Perspectives in biodegradation of pesticides

The knowledge described so far about the genes and enzymes necessary for pesticide degradation and the description of the pathways leading to its mineralization could allow genetic manipulation of microbes to enhance the bioremediation processes for these compounds. Molecular Biology tools provide the development of novel experimental approaches to find and identify novel pesticide-degrading genes. The bottleneck for finding new tools for pesticide bioremediation is that most microorganisms cannot be studied due to culturing limitations. There are estimations that around 99% of microorganisms are uncultivable using standard cultivation methods and therefore not accessible for finding useful genes or enzymes (Lorenz & Eck, 2005; Xu, 2006). To cope with this limitation, the recent development of metagenomic technologies has provided insights about the microbial genetic information available in environmental samples, independent of cultivability. Metagenome is the total biotic genome directly isolated from natural environments, and the power of metagenomics is the access, without prior sequence information, to the so far uncultured majority of microorganisms.

Sequence information from metagenomes can provide two types of approaches for the bioremediation of pesticides. On one instance, biodiversity of contaminated environments can be monitored in order to assess the presence of pesticide degrading bacteria and fungi for biostimulation strategies. The most used molecular markers for this kind of studies are

the ribosomal RNAs, although other markers have also been used (Woese, 1990; Hibbett et al., 2007). Sequence analysis of these molecules permits phylogenetic reconstructions that describe the structure and composition of the microbial population in a determined habitat thus identifying potential microbial species for bioremediation (for example presence of bacteria from genera known to degrade pesticides).

The other approach consists of searching directly for genes or enzymatic activities involved in pesticide degradation. Several successful cases have been reported for esterase activities capable of hydrolyzing organophosphates and pyrethroids (Li et al., 2008; Zhang et al., 2009). In these reports the screenings were performed using substrates that upon hydrolysis produce a distinctive color, thus detecting hydrolases not previously described. The bottleneck of this approach, however, is that the genes must be expressed in a heterologous host (normally *Escherichia coli*), thus missing proteins that need complex posttranslational modifications, eukaryotic genes (that possess introns and promoters that are not recognized in bacteria) or distantly related genes whose promoters will not function in *E. coli*. Other hosts such as yeasts, or the use of expression vectors might be used to overcome some of these limitations.

Another possible strategy is to design degenerate oligonucleotides based on known gene sequences to amplify the desired gene using the metagenome as template DNA (Rose et al., 2003). This strategy will yield genes related to those that have been already described but overcomes the expression step limitation and thus is especially suitable to look for eukaryotic genes (although for further heterologous expression it will be still necessary to obtain the cDNA and use expression vectors).

## 6. The immobilization of microorganisms for massive pesticides degradation

Cell immobilization has been employed for biological removal of pesticides due to the possibility of maintaining catalytic activity over long periods of time (Richins et al., 2000; Chen & Georgiou 2002; Martin et al., 2000). Cell immobilization consists of restricting cellular mobility within a defined space, thereby retaining catalytic activity.

Whole cell immobilization has been shown to have remarkable advantages over conventional biological systems using free cells, such as high cell density, avoidance of cell washout even at high dilution rates, easy separation of cells from the reaction system, repeated use of cells, and better protection of cells from harsh environments. With these advantages, immobilization of microorganisms has been applied in many areas including wastewater treatment and remediation of toxic chemicals. Comparing immobilized cell systems with conventional free cell systems, the productivity obtained with immobilized cells is considerably higher. One obvious reason for this is the high cell density maintained in the reaction system. Some research has suggested that this higher productivity results from cellular or genetic modifications induced by immobilization. Evidences indicating that the immobilized cells are much more tolerant to perturbations in the reaction environment and less susceptible to toxic substances make immobilized cell systems particularly attractive for treatment of toxic substances like pesticides (Ha, 2005).

Immobilization of microorganisms has been applied in many areas including wastewater treatment and remediation of toxic chemicals from this technique generally provides several advantages over cultures using suspended cells that include greater cellular content in the support, enhanced cellular viability (weeks or months) and greater tolerance to high concentrations of pollutants. However, the main limitations to this method are low oxygen

diffusion and interference by the materials used as the support (Martin et al., 2000; Georgiou et al., 2005). Encapsulation and biofilm formation methods are commonly used in environmental contexts. Alginate, *k*-carragenin and polyvinyl alcohol are used as supports for immobilization; ceramics, diatomaceous earth and porous rocks are used for biofilm formation (Karamanev et al., 1998; Davey & Toole 2000; Watnick & Kolter 2000).

In order to have a strategy for the treatment of pesticide wastes, we are also making efforts to build bioreactors with bacteria and/or immobilized yeast cells.

As was mentioned above, there are different materials for the immobilization of cells, but we have looked for some materials that might be economic and with different advantages to design a reactor that can be used on big scale. Tezontle is a volcanic rock that is highly porous. It provides a great contact surface and it can be sterilized and reused. The presence of micropores allows the establishment of bacterial micro colonies. The immobilization method with this material is based on the colonization of the tezontle micropores through the formation of a biofilm.

For the formation of the biofilm, tezontle rocks are crushed to obtain particles of about 3 mm. Tezontle is autoclaved in an intermittent way, at 121°C for 20 minutes, leaving it rest for 24 hours. Then, the microorganisms are planted to allow the formation of the biofilm. Subsequently, a current with the pesticides wastes is passed through to allow the contact with the immobilized microorganisms, so this way the biodegradation can be executed. This strategy has been really efficient and is a tool that can be used for the degradation of pesticides wastes (Yañez-Ocampo et al., 2009 & Yañez-Ocampo 2009).

## 7. Ecotoxicology studies

Ecotoxicology is a toxicology discipline that was proposed by Truhaut in 1969 and studies the adverse effects of toxic substances on ecosystems. This is done by the analysis of the exposure routes, the entry to the organism and the harmful effects on individuals, populations and communities, their way of action, such as the prevention or combat of their harmful effects. A toxic substance is that one that after its penetration in the organism causes immediately, or later on, the suppression of any function of the organism or death.

Soil pollution by pesticides represents a worldwide scale problem, that is why it is necessary to deal with it with an Ecotoxicological point of view, this discipline represents an useful tool to study the destiny and effects of pesticides in the environmental compartments (soil, water, air) and it has the objective to explain the cause and anticipate possible risks of pollution of environmental compartments, and the toxic effects (mortality, immobility, growth and enzymatic inhibition, among others) that pesticides may cause. Different markers have been used (lethality, enzymatic activity ACE, loss of weight and behavior) to prove the pesticides toxicity in laboratory conditions. For their study all the levels of biological organization can be used, from molecules, tissues, organisms and communities, with the purpose of evaluate the pollutant effects. The annelids (oligochaeta), which include the earthworms, are key species in the earth ecosystems that is why they have been widely used as biomarkers in enzymatic studies (acetylcholinesterase) and in studies about the behavior depending of the specific composition of the communities, the competition among species and the digging of galleries (Lavelle *et al.* 1997, Capowiez 2000).

In our working group, the earthworms have been used to measure the effects of pesticides when they are in the soil, as well as the effects of the products that are obtained after the pesticide degradation (Olvera-Velona et al., 2008a).

## 8. Behaviour of pesticides in agricultural soils

The study of pesticides behavior in the soil is really interesting, because it is a heterogeneous, complex and dynamic system, in which different reactions (chemical and biochemical) take place and also it plays a role as receptor of polluting substances. The liquid and gaseous phases in the soil are the main way of transport of the soluble compounds. The solid phase is the main site where accumulation and transformation (chemical or biochemical) of contaminating substances occurs. The pesticides can be directly incorporated into the soil by surface application on the crops, injection, or inadequate aspersion techniques or indirectly through plant leaves.

When the pesticide enters in contact with the soil, sorption is the first process, including adsorption/desorption phenomena. The first one permits fixation of the compounds to the soil particles; the last one releases the pesticide into soil solutions. The sorption process is related very with the persistence and pesticide degradation, because the physicochemical and biological characteristics of soils play a key role (Madrigal-Monárrez et al., 2008).

## 9. Pesticides sorption in soils

The extensive use of pesticides in agriculture has resulted in the widespread distribution in the environment. Organophosphate insecticides are increasingly used in agriculture as a substitute for organochlorine and carbamate insecticides, because of their higher efficiency and lower persistence. However, it is necessary to evaluate the risks and toxicity of these compounds and their degradation products into the groundwater, and their movement through the food chain (Pehkonen & Zhang, 2002).

The pesticide fate in soils strongly depends on adsorption-desorption phenomena. These processes influence the composition of the soil solution, and knowledge and understanding of them is important to accurately predict the mobility and bioavailability of these chemicals in soils; this would permit to limit their impact on non-targeted organisms and ecosystems. Pesticide availability in soil can be evaluated using indirect and direct methods. The most common indirect method to characterize pesticide availability is by using a partition coefficient ( $K_d$ ). The pesticides with high soil-liquid partition coefficients ( $K_d$  values) are more strongly adsorbed to soils and transported in the soil solution. In contrast, pesticides with low  $K_d$  values are only weakly adsorbed to soils and are more likely to leach, as they are more water-soluble and hence exhibit greater mobility in the soil solution. However, sorption-desorption processes are complex and cannot be described by a single value, assuming instantaneous totally reversible sorption (Pignatello, 2000). Desorption processes control the release rate of pesticide into the soil solution, and thus have a major control of availability and bioavailability to soil organisms (Weber et al., 1993). Increasing pesticide contact time or ageing affects sorption and desorption processes, and generally desorption decreases with the residence time in soil. As a consequence, the availability of pesticide for transport or uptake by soil organisms decreases with time (Gevao et al., 2001; Mamy & Barriuso, 2007).

The sorption and degradation are influenced by physicochemical properties of the soil [such as pH and organic carbon (OC) content], biological properties (activity and distribution of microorganisms), and environmental conditions that control soil temperature and moisture content. Both route and rate of degradation also depend on properties of the chemicals. For ionized molecules, mineralogical composition and soil pH are key parameters, whereas

sorption of neutral compounds is mostly governed by the soil organic matter (Cooke et al., 2003).

These generally accepted rules have been obtained in studies considering large number of soil types and origins. Nevertheless, the role of soil characteristics is less well documented for tropical soils, compared with temperate soils (Zheng et al., 1994; Zheng & Cooper, 1996; Wauchope et al., 2002; Oliver et al., 2005).

Several pedological factors are known to regulate sorption and desorption processes (Weber et al., 2004). For molecules, mineralogical composition and pH are key parameters. The relations between sorption and soil type characteristics strongly depend on molecular properties, such as electric state and polarity (Barriuso & Calvet, 1991). The combination of effects of soil organic matter and of mineral constituents, such as smectite-type clays and amorphous clays-allophanes, usually increases the sorption of neutral molecules. They have a higher cation exchange capacity than other soil colloids, such as clays, and thus play an important role in adsorption reactions and determining fate of pesticides in the environment (Cooke et al., 2003).

The studies about pesticide fate in soil are complex due to physical, chemical, and biological interactions occurring simultaneously. In this way, it has been suggested an integral view relating sorption and transformation (degradation or mineralization), considering the physicochemical properties of pesticides and soil. There are experimental tools, such as soil microcosms (experimental devices with nominal variables and standard conditions), that help to explain the fractioning (or mass balance) of pesticides into the liquid or solid soil phases.

Our group has performed work in assays carried out in microcosms with parathion ( $^{14}\text{C}$ -U-labeled), in two crop soils (andisol and vertisol), after an incubation time of 32 days (Olvera-Velona et al., 2008b). Some of the results are outlined below.

**Soluble fraction.** This pesticide fraction is easily available for microorganisms; and thus, it can be easily biodegraded. This is the fraction with the highest mobility and can be lixiviated into the aquifer mantles. The aqueous solubility of pesticides is the most related property with mobility and lixiviation. Pesticides with aqueous solubility higher than 30 mg/L represent a potential lixiviation risk (Xing & Pignatello, 1996). Adsorption coefficient ( $K_d$ ) is another property related with the soluble fraction of pesticide in the soil.  $K_d$  values below 30 L/Kg indicate lesser affinity to the soil particles and higher affinity for the liquid phase (in solution) of the soil. Microcosms tests also supplied us information about parathion fractioning. The soluble fraction was 2 and 3% at 32 days, in vertisol and andisol soils, respectively.

The  $K_d$  values for parathion were  $74.9 \pm 0.6$  L/Kg and  $38.6 \pm 0.9$  L/Kg in andisol and vertisol respectively, and when related with solubility (11 mg/L), we can infer that parathion has higher affinity for the solid phase in soil and lixiviation risk is low. The result indicates that only a small fraction (2-3%) can be found in the liquid soil phase in soluble form.

**Adsorbed fraction.** This is the pesticide fraction that is potentially available, because it is retained in the solid particles of soil. Surely, this is the pesticide fraction with highest interaction with soil. This fraction can be studied by measuring the adsorption coefficient ( $K_d$ ); with the  $K_d$  values we can estimate the amount of pesticide adsorbed by the solid phase of soil.  $K_d$  values above 200 L/Kg are related to high affinity with soil particles. Spark & Swift (2002) reported that organic matter is one of the most related parameters with adsorption process. Weber et al., (2004) reported that soil pedological factors (organic matter and clay minerals) increase pesticide adsorption. Adsorbed fractions from 50 to 46%, in andisol and vertisol soils were found, respectively. The fraction percentages reflected the

affinity for soil organic matter. However, when the organic matter content is below 2% (in the case of vertisol), an affinity for soil inorganic constituents (as smectites and allophan) was found (Worall et al., 1996).

**Strongly bound fraction.** This fraction directs to the pesticide stabilization, and represents the less available or biodegradable forms. This fraction also is known as bound residues. Barriuso et al, (1994) suggested the formation of covalent binding with humic substances; these interactions confer stability to pesticide. The residue formation can be considered an alternative to reduce the lixiviation risk; however, also the availability (and bioavailability) is reduced. Studies carried out by Mordaunt et al., (2005) suggested that, through time and agricultural practices, these residues can be reactivated, pass to the soil solution phase, and be again available. The parathion bound residue formation was 45 and 35% in andisol and vertisol, respectively. These percentages indicated us that a significant proportion of parathion was found inactive in the soil particles (at 32 days).

**Mineralized fraction.** This is the fraction of pesticide that has been transformed by microbes into components, such as  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and  $\text{NH}_4^+$ . Therefore, the mineralization is the suitable process for soil depuration. The metabolic activity of microbial populations and the co-metabolism are key determinants for the process. The microbial adaptation and enzyme production are important factors to catalyze the degradation reactions. The microbial consortia can carry out the degradation or mineralization with high efficiency, because the primary consumers start the degradation process and the secondary ones utilize the metabolic products from the formers and take them to degradation. This can facilitate the growth of primary consumers, by supply of metabolic products (e. g. growth factors), and by elimination of toxic compounds through co-metabolism. The mineralized fraction of parathion was 8 and 9% in andisol and vertisol soils, respectively. We consider that parathion mineralization in both soils was low. By relating this fraction with the soluble one (available), which was 2 and 3% in andisol and vertisol, respectively, results are logic. However, we also need consider the pesticide properties such as molecular structure, concentration, and bioavailability, because they are closely related with the mineralization process.

## 10. Conclusion

The use of chemical pesticides has brought benefits such as the increment of agricultural production, soil productivity and products quality, which is reflected in economical benefits, vector disease control and in general, in public health. However, due to only 10 per cent of applied pesticides reach to the target organism, a high percentage is deposited on non target areas (soil, water, sediments) and impacts to non target organism such wild life, besides affecting public health. Due to the extensive pesticides use, currently there are polluted sites with these compounds (mainly soils), besides of the production of great amounts of pesticide wastes, stored obsolete pesticides and empty containers. For this reason, it is necessary to generate strategies for waste treatment and/or for the bioremediation of polluted sites. The biological treatment is an important technology from an economical and environmental point of view. Currently, the use of native or genetically modified organism to degrade or remove pesticides has emerged as a powerful technology for in situ remediation. There are reports of different organisms (bacteria, algae, yeasts, fungus and plants), characterized in relation to their genome and the enzymes that they produce, that can be used for waste treatment or bioremediation of soil and water. In this

last process, it is necessary to considerate the soil composition, which is the responsible of the final destination of these compounds. Another important aspect is the ecotoxicology study, which will help to prevent the native organisms of soil and water.

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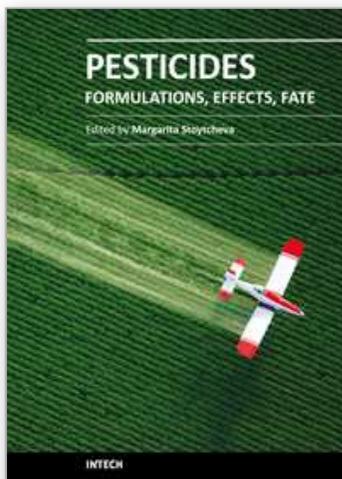
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## **Pesticides - Formulations, Effects, Fate**

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This book provides an overview on a large variety of pesticide-related topics, organized in three sections. The first part is dedicated to the "safer" pesticides derived from natural materials, the design and the optimization of pesticides formulations, and the techniques for pesticides application. The second part is intended to demonstrate the agricultural products, environmental and biota pesticides contamination and the impacts of the pesticides presence on the ecosystems. The third part presents current investigations of the naturally occurring pesticides degradation phenomena, the environmental effects of the break down products, and different approaches to pesticides residues treatment. Written by leading experts in their respective areas, the book is highly recommended to the professionals, interested in pesticides issues.

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