BIOLOGICAL ASPECTS INVOLVED IN THE DEGRADATION OF ORGANIC POLLUTANTS

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ABSTRACT

Worldwide use of pesticide has increased dramatically during the last two decades. As a consequence, pesticide residues and their transformation products are frequently found in groundwater and surface waters. This review summarizes information about polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), some chlorophenols; it mainly empathizes on pesticides, their incorporation into the environment, microorganisms involved in their degradation and, some physico-chemical aspects of pesticides behavior in soils. Details about residues of pesticides in groundwater and superficial water found in some foreign countries and in Chile are reported, as well. The fungal degradation of organic pollutants (xenobiotics) is considered as an effective method to remove these pollutants from the environment by a process which is currently known as bioremediation. Therefore, the degradation of pesticides by soil microorganisms with particular attention to white-rot fungi is also addressed. Finally, a simple and effective system (biobed) to minimize environmental contamination from pesticide manipulation, especially when filling the spraying equipment, a typical point source of contamination, is presented here.

Keywords: Biodegradation, white-rot fungi, pesticides, biobeds.

INTRODUCTION

In the last few decades, highly toxic organic compounds have been synthesized and released into the environment for direct or indirect application over a long period of time. Pesticides, fuels, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorophenols, and dyes are some of these types of compounds. Some synthetic chemicals are extremely resistant to biodegradation by native flora (Rochkind-Dubinsky *et al*., 1987) compared with the naturally occurring organic compounds that are readily degraded upon introduction into the environment. Therefore, hazardous wastes and chemicals have become one of the greatest problems of modern world-wide society.

Biological decomposition of pesticides is the most important and effective way to remove these compounds from the environment. Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule (Raymond *et al*., 2001; Wiren-Lehr *et al*., 2002). Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide degraders (De Schrijver and

De Mot, 1999). Fungi generally biotransform pesticides and other xenobiotics by introducing minor structural changes to the molecule, rendering it nontoxic; the biotransformed pesticide is released into the soil, where it is susceptible to further degradation by bacteria (Gianfreda and Rao, 2004).

Among white-rot fungi, *Phanerochaete chrysosporium* and *Trametes* versicolor have become the most commonly used model organisms in lignin biodegradation studies due to their good ligninolytic properties, fast growth, and easy handling in culture (Gold and Alic, 1993; Mougin *et al*., 2002). The extracellular peroxidase enzyme systems employed are non-specific (Evans *et al*., 1994), and have been implicated in the degradation by white-rot fungi of a variety of contrasting aromatic xenobitics, including chlorophenols, pesticides and dyes (Pointing, 2001; Bending *et al*., 2002; Tortella *et al*., 2005; Rubilar *et al*., 2007), although the mechanisms involved are not clearly related to the ligninolytic potential of these organisms. As a way of not altering natural ecosystems, the trend in the last years has been the search and utilization of native fungi with ligninolytic potential as well as the evaluation of technologies that allow optimizing the conditions of degradation of organic recalcitrant compounds. In this respect, the white-rot fungus *Anthracophyllum discolor* Sp4, a Chilean native fungus, presents a high ligninolytic activity, mainly manganese peroxidase (MnP) activity, and therefore it has a great potential for xenobiotic degradation (Tortella *et al*., 2008).

PERSISTENT ORGANIC POLLUTANTS

Persistent organic pollutans (POPs), most of them with hydrophobic properties (HOCs), include simple aromatic compounds such as benzene, toluene, ethylbenzene and xylenes (BTEX), polycyclic aromatic hydrocarbons (PAHs), including naphthalene, phenanthrene and benzo[α]pyrene, and polychlorinated biphenyls (PCBs). These compounds are poorly soluble in water, and resistant to biological, chemical, and photolytic degradation (Semple *et al*., 2003). Among them, compounds like pesticides, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) arrive to the soil from different sources and have high toxicity toward humans, plants, and animals. Soil microbial and biochemical activities are sensitive to several natural and human induced changes and may provide a helpful tool to assess soil status, its quality, and its productivity. In respect to pesticides, they may enter the soil by application events, atmospheric wet or dry deposition, foliar wash-off, and accidental spills onto soil surface or into the soil profile. Similarly, PAHs may enter the soil from several sources, such as incomplete combustion of coal, oil, petroleum, and wood; petrochemical industries; dismissed industrial sites; and effluents from vehicle and equipment cleaning and maintenance (Gianfreda and Rao, 2008).

When an organic component enters the soil, it can be subjected to transfer processes that relocate the substances without altering their structure or degradation processes. In soil, an organic compound can be weakly or strongly associated to inorganic and organic colloids through adsorption mechanisms (Bayley and White, 1970; Senesi, 1992; Cea *et al*., 2005; Cea *et al*., 2007a). It can be adsorbed by plant roots or volatilized, depending on the soil zone where it is located, and on its chemical and physical properties (Singh *et al*., 2004; Meade and D'Angelo, 2005; Kremer and Means, 2009). If the organic compound

accumulates on soil surface, it can undergo photodecomposition and the process will be strongly influenced by intrinsic and extrinsic soil properties as well as by the inherent chemical structure of the compound.

The removal of POPs and HOCs from contaminated sites is a widespread environmental problem today. Bezama *et al.* (2008) reviewed remediation technologies for organochlorinated contaminated sites in developing countries. The authors present relevant information about the distribution of polychlorinated biphenyls (PCBs) and their quantification in different regions of Chile, soil and water contamination, and treatment technologies to eliminate the contaminants.

Synergistic solubilization of polycyclic aromatic hydrocarbons by mixed anionic-nonionic surfactants has been studied by Zhu and Feng (2003). Chu and Chan (2003) studied the mechanism of the surfactant-aided soil washing system for hydrophobic and partial hydrophobic organic compounds. Other works have aimed at the effect of rapeseed oil on the degradation of polyclyclic aromatic hydrocarbons in soils by *Rhodococcus wratislaviensi* (Pizzul *et al*, 2007) and with degradation of polycyclic aromatic hydrocarbons by free and nanoclay-immobilized manganese peroxidase from *A. discolor* (Acevedo *et al*., 2010).

Pesticide contamination

Worldwide pesticide usage has increased dramatically during the last two decades, coinciding with changes in farming practices and the increasing intensive agriculture. This widespread use of pesticides for agricultural and non-agricultural purposes has resulted in the presence of their residues in various environmental matrices. Pesticide contamination of surface waters has been well documented worldwide (Table 1), and constitutes a major issue that gives rise to concerns at local, regional, national and global scales (El-Nahhal *et al*., 1997; Planas *et al*., 1997; Fatoki and Awofolu, 2005; Westbom *et al*., 2008). Pesticides such as atrazine, isoproturon, diuron and mecoprop are the main herbicides found in groundwater in the UK, whereas atrazine, alachlor, metolachlor, etc. were detected in ground water in other European countries and in America and Africa (Table 1).

In Chile, several pesticides are used for different proposals and are permitted currently by the legislation. Some pesticides that are used in great quantities for crop protection are chlorpyrifos, atrazine, dimethoate and isoproturon (www.sag.cl). The main features of these pesticides are presented in Table 2. Many pesticides compounds are hydrophobic or moderately hydrophobic with a complex chemical composition that is very different from hydrocarbons and their derivatives. Atrazine is a hydrophobic pesticide frequently found in monitoring pesticides in aquifers of different countries (Hallberg, 1989; Leistra and Boesten, 1989; Laabs, *et al*., 2002).

Few studies have been developed in relation with soil, water, and sediment contamination with pesticides in Chile (Table 3). Henriquez *et al*. (2006) reported that the pesticides Aldrin DDT and dieldrin were found in 41.2 %, 32.4 % and 29.4%, respectively, of the soil samples in Ñuble region. Barra *et al*. (2001) reported that organochlorine pesticides were found in sediment cores from four Chilean lakes in Bío-Bío Region. High concentrations of PCBs, lindane and pp′-DDT were detected by Focardi *et al*. (1996) in different locations of the Biobio river basin (central Chile)– located about 5 km from San Pedro lagoon.

Table 1. Residues of pesticides in groundwater and superficial water found in some countries.

Country	Detected pesticides	Levels	Water	Ref
Portugal	Lindane, atrazine, simazine,dimethoate, metribuzin, endosulfan, prometryn, metolachlor	0.22–17 μg L^{-1}	Groundwater	Barcelo (1991)
	Quinalphos (28.8%), Paraquat (27,9%)	*		Teixeira *et al.*, (2004)
Netherlands	Atrazine, simazine, dieldrin, propazine and lindane ($\beta+\gamma$-HCH)	100-200 ng L^{-1}	Groundwater	Maanen *et al.*, (2001)
	A broad list of pesticides (27)	**27% ***11%	Groundwater	Schipper *et al.* (2008)
Greece	Lindane (γ-BHC), chlorpyrifos, propachlor,	0.005-0.01 μg L^{-1}	Groundwater	Karasali *et al.* (2002)
Canada	Alachlor, metalaclhor, atrazine, metribuzin, cianazina.	0.17–0.34 μg L^{-1}	Superficial	Goss *et al.* (1998)
England	Lindane ($\alpha+\beta$-HCH), heptachlor, Aldrin, γ-Chlordane, endosufan, dieldrin, endrin, 2,4'-DDT, etc.	5.5-160 ng L^{-1}	Superficial	Fatoki and Awofolu (2005)
South Africa	Lindane ($\alpha+\beta$-HCH), heptachlor, aldrin, γ-Chlordane, endosufan, dieldrin, endrin, 2,4'-DDT, etc.	6–80 ng L^{-1}	Superficial	Fatoki and Awofolu (2005)
Brazil	Alachlor, atrazine, chlorothalonil, endosulfan, simazine, metribuzin, monocrotofos, malathion, chlorpyrifos, metribuzin, etc.	0.001-0.174 μg L^{-1}	Superficial, River, Lakes	Laabs, *et al.* (2002)
Spain	Atrazine, desethylatrazine, simazine, desethylsimazine, metolachlor, desethylterbuthylazine, terbuthylazine, metalaxyl	Up to 0.63 Up to 2.46	Superficial Groundwater	Hildebrandt *et al.* (2008)
Hungary	Acetochlor, atrazine, carbofuran, diazinon, fenoxycarb, metribuzin, phorate, prometryn, terbutryn, and trifluralin	**59%	Superficial	Maloschik *et al.* 2007
USA	Data from 10 years of study (25 pesticides)	-	Groundwater Streams	Gilliom, 2007

* Represent positive cases of intoxication;
** Percentage of the samples containing pesticide;
*** Percentage of samples exceeding 0.1 μg L^{-1}.

Table 2. Characteristics of the pesticides with major frequency of use in Chile.

Activity	Classification	$t_{1/2}$ días	Water Solubility	Koc	Sales* (%)
Insecticides-Acaricides		264			
Mineral oil	-				36.94
Chlorpyrifos (II)	Organophosphate	60-120	1.4 mg L^{-1}	125	9.86
Cartap hydrochloride (II)	Carbamate	3	200 g L^{-1}	ni	7.06
Diazinon (I)	Organophosphate	9	60 mg L^{-1}	413-760	5.21
Azinphosmethyl (I)	Organophosphate	87	28 mg L^{-1}	882	3.9
Methamidophos (I)	Organophosphate	4	200 g L^{-1}	1-5	3.52
Esfenvalerate (I)	Pyrethroid	44	0.002 mg L^{-1}	5300	3.37
Methyl bromide (I)	Organobromine	55	13200 mg L^{-1}	39	1.37
Dimethoate (II)	Organophosphate	2-4	25 g L^{-1}	16-52	0.97
Carbofuran (I)	Carbamate	30-60	320 mg L^{-1}	17-28	0.9
Fungicides-bactericides					
Sulphur	Sulphur				62.21
Copper oxychloride (III)	Copper	10.000	1.19 mg L^{-1}	-	2.9
Chlorothalonil (IV)	Chloronitrile	5-36	0.9 mg L^{-1}	300-7000	1.84
Captan (IV)	Dicarboximide	1	3.3 mg L^{-1}	97	0.6
Carbendazim (IV)	Benzimidazol	25-500	8 mg L^{-1}	200-250	0.06
Herbicides					
Glyphosate (IV)	Organophosphate	3-60	12 g L^{-1}	ni	58.83
MCPA, amine salt (II)	Methylphenoxyacetic	< 7	734 mg L^{-1}	ni	2.05
Paraquat (I-II)	Bipyridin	3000-5000	700 g L^{-1}	1000000	3.44
Atrazine (III)	Triazine	35-50	33 mg L^{-1}	39-155	1.8
2,4-D / picloram (II)	Phenoxyacetic/AcPyridincarboxilic	7 /30-300	500 mg L^{-1}	20-60	0.43
Simazine (IV)	Triazine	70-110	6.2 mg L^{-1}	103-377	2.67
Metsulfuron methyl (IV)	Sulfonylurea	10	2790 mg L^{-1}	40	2.68
Diuron (II)	Phenylurea	90-180	42 mg L^{-1}	400	0.38
Diclofop-methyl	Phenoxypropionic	23-123	0-8 mg L^{-1}	16.000	0.06

*Sales in 2006. Information obtained from SAG (2006).; ni: no information; I: Extremely dangerous; II: Moderately dangerous; III: Scantily dangerous; IV: Not dangerous

Table 3. Residues of pesticides in water and sediments detected in Chile.

Place	Detected pesticides	Levels	Sample	Ref.
Aconcagua River	Lindane, diazinon, oxifluorphen, dicofol, azinfos-methyl, metabenzotiazuron, lenacil , diflubenzuron, atrazine	0.1-1.12 $\mu g\ L^{-1}$	Water	Baéz *et al.* (1996)
Traiguen River	Simazine, 2,4-D, pichloram, hexazinone, carbendazim	0.2-9.7 $\mu g\ L^{-1}$	Water	Palma *et al.* (2004)
San Pedro lake Lleu-Lleu, Icalma	Heptachlor, aldrin, endrin, pp´-DDT, pp'-DDE, pp'-DDD	1.68-0.89 $ng\ g^{-1}$	Sediments	Barra *et al.* (2001)
Chillan River	Heptaclor, endrin, endosulfan, metoxichlor	35-58 $ng\ L^{-1}$	Water	Cooman *et al.* (2005)
Limari River basin	2,4 D, Aldicarb, atrazine + N-dealkyl metabolites, captan, carbofuran, chlorothalonil, cyanazine, dimethoate, diclofop-methyl, parathion, pentachlorophenol, simazine, trifularine	1-0.05 $\mu g\ L^{-1}$	Water	DGA (2004)

Pesticides in soil

How long the pesticide remains in the soil depends on how strongly it is bound by soil components and how readily it is degraded, and it also depends on the environmental conditions at the time of application, e.g., soil water content (Arias-Estévez *et al.*, 2008). A complete review about the mobility and degradation of pesticides in soils and groundwater resources pollution was provided by Arias-Estévez *et al.*, 2008. The review shows the influence of the physical and chemical characteristics of the soil system, such as moisture content, organic matter and clay contents, and pH, on the sorption/desorption and degradation of pesticides and their access to groundwater and surface waters. Future prospects to develop microbially derived pesticides, and new application techniques not only for reducing the dose, which can be a very effective way to minimize transport and emission, but also to avoid a resistance build-up in target organisms, are also addressed in the review.

The adsorption and mobility of organic pesticides in soils depend on the ionic or neutral character of the molecule, on its water solubility and its polarity as well as on the content and nature of the colloidal fraction of the soil, clay and organic matter (Shawhney and Brown, 1989). Adsorption and desorption of pesticides on soils are the main retention phenomena that determine their transport, transformation, and biological effects in soil environments (Barriuso *et al.*, 1994). For example, atrazine is moderately up to highly mobile in soils, especially where soils have low clay or organic matter content (Barriuso and Calvet, 1992). Atrazine is primarily retained on silicate clays by physical adsorption but both physical adsorption and chemisorption

contribute to the retention of atrazine by soil organic matter (Laird *et al.*, 1994). Rama and Ligy (2008) evaluated adsorption and desorption characteristics of lindane, methyl parathion and carbofuran in soil and they reported that clay content and organic matter played a significant role in pesticide adsorption and desorption processes. Alister *et al.* (2008) studied the persistence, adsorption and movement of flumioxazin in soil in four Chilean vineyard production areas. The authors found that flumioxazin is an herbicide with low environmental risk owing to its short DT_{50}, reduced soil residues 3 months after application and low effective dose.

Insecticide adsorption by soils and lake sediments has been shown vary with type of pesticide, pH, temperature, clay and organic matter content (El-Nahhal *et al.*, 2001). Clausen and Fabricious (2002) studied atrazine, isoproturon, mecoprop, 2,4-D and bentazone adsorption onto iron oxides. They found that acidic pesticide adsorption is strongly affected by pH and $CaCl_2$ concentration in solution. Boivin *et al.* (2005) evaluated the adsorption and desorption of trifluralin, 2,4-D, isoproturon, atrazine and bentazone in thirteen agricultural soils. They reported that atrazine, isoproturon and trifluralin adsorption on soil was correlated to soil organic matter content and bentazone adsorption was governed by soil pH.

According to Taylor and Spencer (1990), the two main environmental factors that affect pesticide behavior in soil are moisture and temperature, with moisture having a more significant relative weight than soil temperature. However, Bromilow *et al.* (1999) observed that soil moisture did not influence the degradation rate of flutriafol, epoxiconazole, propiconazole, triadimefon and triadimenol fungicides. With their experiments, the authors

identified an opposite relationship between soil temperature and the degradation rate of these five fungicides. Awasthi and Prakash (1997) evaluated the fate of chlorpyrifos in soils under different moisture regimes. The major environmental factors that influenced chlorpyrifos loss in soil were moisture, pH, organic matter and clay content. Chlorpyrifos was degraded rapidly in all air-dry soils and slightly more slowly in soils at field capacity and/or under submerged conditions.

The temperature is another factor that can affect pesticide behavior in soil. Paraíba and Spadotto (2002) reported that atrazine and lindane behave as hazardous chemicals in sites where soil temperature is low or very low, with top soil temperature less than 20 °C. Brücher and Bergström (1997) determined that linuron sorption to three different agricultural soils was dependent on temperature. Paraiba *et al.* (2003) reported that soil temperature affected thirty pesticides' leaching potential into groundwater and indicated that contamination by these compounds varies with different climatic conditions.

Many investigators have noted that pH plays an important role in the adsorption of compounds with acidic functional groups on activated carbon and soil because the neutral and ionic forms display very different adsorption properties (Kookana and Rogers, 1995; Diez *et al.*, 1999; Diez *et al.*, 2005; Cea *et al.*, 2005; Cea *et al.*, 2007a). However, chlorophenol sorption in soil has been generally described assuming hydrophobic partitioning of the neutral species to soil organic matter (DiVicenzo and Sparks, 2001). In a variable-charge soil (Andisol), Diez *et al.*, 1999 demonstrated that the adsorption of phenolic compounds from kraft mill effluent increased with decreasing pH, possibly as a consequence of electrostatic

repulsions between the organic compound and the resulting negative surface charge as pH increases. The same behavior was observed by Cea (2006) when evaluating the effect of soil pH of an Andisol from Southern Chile on three chlorophenols frequently found in industrial processes (Figure 1).
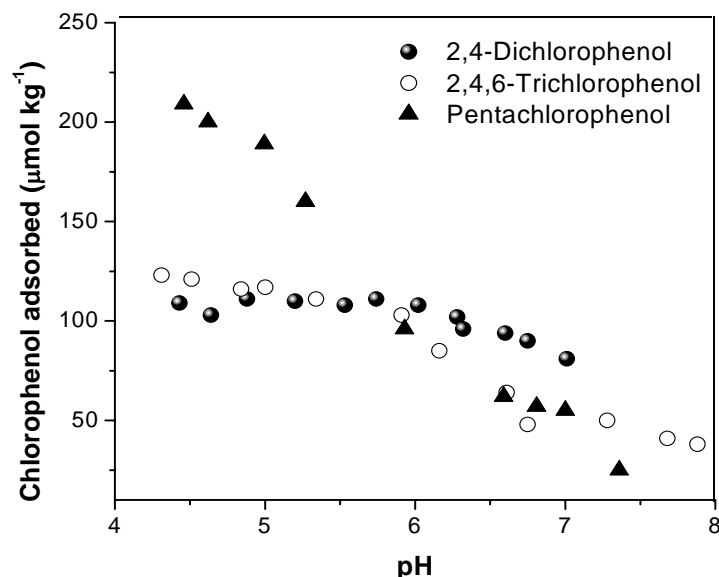
Figure 1. Effect of soil pH on chlorophenols adsorption at 25 °C, in KCl 0.1 mol L^{-1} (Cea, 2006).

An exhaustive review on the current state of knowledge regarding the formation and biological/environmental significance of bound pesticide residues in soils was provided by Gevao *et al*. (2000). The authors defined various terms used in the discussions identifying the types/classes of pesticides which may be added to soil and interact with it. They considered various soil properties and aspects of land management which will influence nature and degree of the soil-pesticide association, and discussed the possible physical and chemical binding mechanisms. They also took into account the role of microorganisms and other forms of soil biota in bound residue formation and the bioavailability of soil-borne pesticide residues.

DEGRADATION OF PESTICIDES BY SOIL MICROORGANISMS

Several works have reported that native microorganisms from soil and sediment are capable of degrading pesticides. Eizuka *et al*. (2003) evaluated ipconazole (triazole fungicide) degradation by soil microorganisms. They reported that bacteria, Actinomycetes and fungi were responsible for the degradation of this compound. Singh *et al*. (2008) isolated a native fungal strain from corn field soil able to use atrazine as source of

nitrogen. The fungus degraded 44% of atrazine in 20 days. Chlorpyrifos has been reported to be resistant to enhanced degradation which was attributed to the antimicrobial activity of 3,5,6-trichloro-2-pyridinol (TCP) (Racke *et al.*, 1988). However, Venkata Mohan *et al.* (2004) studied the degradation of a chlorpyrifos contaminated soil using native mixed microflora in slurry bioreactor at 3000 µg/g, 6000 µg g^{-1} and 12000 µg g^{-1}. They found that 91%, 82% and 14% of chlorpyrifos was respectively degraded after 72 h. Yu *et al.* (2006) isolated and characterized a fungal strain capable of degrading chlorpyrifos utilizing the pesticide as sole carbon and energy. They reported that the fungus was able to degrade >80% of chlorpyrifos.

Pesticides are usually applied simultaneously or one after another for crop protection, and this type of pesticide application often leads to a combined contamination of pesticide residues in the soil environment. A laboratory study was conducted to investigate the influence of chlorothalonil on chlorpyrifos degradation and its effects on bacterial, fungal, and Actinomycete soil populations (Chu *et al.*, 2008). Chlorpyrifos degradation was not significantly altered by its combination with chlorothalonil. However, the inhibitory effect of chlorpyrifos on soil microorganisms was increased by its combination with chlorothalonil, and the increase was related to the added chlorothalonil levels. Compared with those in the controls, the populations of bacteria, fungi, and Actinomycetes were significantly reduced by chlorpyrifos alone, and the inhibition increased with chlorothalonil addition. The results suggested that combined effects should be taken into account to assess the actual impacts of pesticide applications.

A review of Briceño *et al.* (2007) shows that various bacteria and fungi present in soils has the capacity to degrade or mineralize several pesticide groups. The studies show that addition of organic amendment and nutrients can affect mainly the adsorption, movement, and biodegradation of pesticides.

Repeated application of pesticides in the same field for a certain number of years developed an active microbial population in soil with the ability to degrade determined compounds (Hernández *et al.* 2008). Pesce and Wunderlin (2004) reported the biodegradation of lindane by a native bacterial consortium isolated from contaminated soil. These authors reported that *B. thiooxidans* and *S. Paucimobilis* degraded lindane after 3 days of aerobic incubation. Mercadier *et al.* (1997) reported that 50% of fungicide iprodione was degraded by bacterial isolated from soil as *Pseudomonas* sp. Atrazine, monocrotophos, alachlor and 4-chlorophenol have been degraded also by soil microorganism (Westerberg *et al.*, 2000; Bhadbhade *et al.*, 2002; Strong *et al.*, 2002). Guerin (1999) studied the natural attenuation of a low mobility insecticide, endosulfan, in low and high level contaminated soil, but the mineralization did not play any important role in natural attenuation of endosulfan by soil microorganisms. Chirnside *et al.* (2007) studied an indigenous microbial consortium isolated from contaminated soils to evaluate its potential to degrade atrazine and alachlor. They determined that the indigenous microbial consortium was capable of degrading both herbicides, but the consortium exhibited a unique degradation pattern being atrazine degradation dependent on alachlor degradation.

Many Actinomycetes can degrade different pollutants, including several pesticides. A review by De Schrijver and De Mot (1999) showed that the genera *Arthrobacter*, *Clavibacter*, *Nocardia*, *Rhodococcus*, *Nocardioides*, and

Streptomyces behave as pesticide degrading Actinomycetes. The metabolic pathway for pesticide degradation by Actinomycetes has not been studied extensively; however, it is known that these microorganisms can produce extracellular enzymes that degrade a wide range of complex organic compounds. A common feature of the aerobic Actinomycetes is the presence of many types of monooxygenases and dioxygenases (Larkin *et al.*, 2005). Most recent studies have isolated and characterized different Actinomycetes capable of degrading pesticides and many strains of this group are proposed to be used for soil decontamination (Benimeli *et al.*, 2003). Benimeli *et al.* (2008) studied the lindane bioremediation ability of *Streptomyces* sp. M7 in soil samples and the pesticide effects on maize plants seeded in lindane-contaminated soil previously inoculated with *Streptomyces* sp. M7. Results showed that *Streptomyces* sp. increased the biomass and concomitantly decreased residual lindane. The activity of this strain was not inhibited by natural soil microbial flora and its growth was not inhibited by high pesticide concentration.

White-rot fungi for persistent organic pollutants degradation

A critical review of the present state of fungal activities on environmental pollutants, fungal diversity, the use of fungi in the degradation of chemical pollutants, enzyme degrading systems and perspectives on the use of fungi in bioremediation and unexplored research was reported by Tortella *et al.* (2005). The authors summarize information from fundamental works that reveal that a wide variety of fungi are capable of degrading an equally wide range of toxic chemicals. The capacity of non-ligninolytic and ligninolytic fungi in the bioremediation of

polycyclic aromatic hydrocarbon (PAHs), benzene - toluene - ethylbenzene - xylene (BTEX), chlorophenols, polychlorinated biphenyl, munitions waste and pesticides have been discussed. Besides, several extracellular enzymes are involved in the metabolism of xenobiotic compounds as well as other factors related to these processes. Pointing (2001) presents evidence for the involvement of ligninolytic enzymes in white-rot fungal degradation of munitions waste, pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, bleach plant effluent, synthetic dyes, synthetic polymers, and wood preservatives.

D'Annibale *et al.* (2005) studied the degradation of aromatic hydrocarbons by white-rot fungi (*P. chrysosporium* NRRL 6361 and *Pleurotus pulmonarius* CBS 664.97) in a historically contaminated soil. They reported a total removal of some PAHs (naphtalene, tetrachlorobenzene, and dichloroaniline isomers, diphenylether and N-phenyl-1-naphtalenamine), and a significant release of chloride ions in fungal-treated soil, in comparison with that recorded in the control microcosm. Both fungi led to a marked soil detoxification, even in the presence of a high residual concentration of the main components of the aromatic hydrocarbons fraction.

A survey of the mutual interactions establishing in a soil among xenobiotic substances with particular reference to pesticides and polycyclic aromatic hydrocarbons and microbial and enzymatic soil activities has been reviewed by Gianfreda and Rao (2008). On the other hand, Rubilar *et al.* (2008) analyzed the presence of chlorophenols in the environment, their main chemical and physical properties, and the main processes in their degradation by white-rot fungi with particular attention to soil environment. The reactions involved in the process, the intermediary products, the

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factors that may affect the fungal transformation of chlorophenols, and the possible applications for environmental purposes of both the whole fungal cells and/or their enzymes as isolated catalytic agents are also addressed. The biological agents involved in the bioremediation of soils contaminated with persistent organic compounds have also been analyzed by Gianfreda *et al*. (2006).

White-rot fungi (Basidiomycetes) are the only known organisms capable of degrading lignin extensively to CO_2 and H_2O in a pure culture. These organisms are able to degrade all the major wood polymers: cellulose, hemicellulose, and lignin (Gold and Alic, 1993). As mentioned above, *P. chrysosporium* and *T. versicolor* are the most commonly used model organisms in lignin biodegradation studies (Gold and Alic, 1993). They possess enzymes, such as lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase, all involved in lignin degradation. The extracellular enzymatic systems employed are non-specific, and are used in the degradation of several xenobiotics, including pesticides (Bending *et al*., 2002).

Degradation of pesticides by white-rot fungi.

Several classes of pesticides as lindane, atrazine, diuron, terbuthylazine, metalaxyl, DDT, gamma-hexa-chlorocyclohexane (g-HCH), dieldrin, aldrin, heptachlor, chlordane, lindane, mirex, etc. have been degraded at different extent by white-rot fungi (Kennedy *et al*., 1990; Hickey *et al*., 1994; Mougin *et al*., 1994; Singh and Kuhad, 1999; Bending *et al*., 2002; Quintero *et al*., 2007). Jauregui *et al*. (2003) reported that *Bjerkandera adusta*, *Pleurotus ostreatus* and *P. chrysosporium* depleted 50 to 96% of terbufos, azinphos-methyl, phosmet and tribufos after four-

day exposure to the pesticides. MCPA and bentazon were degraded by *P. chrysosporium* at 65% and 75% respectively in 20 days (Castillo *et al*., 2001). Rubilar *et al* (2007) and Tortella *et al*. (2008) reported that the native Chilean white-rot fungi, *A. discolor* can degrade and grow in the presence of chlorinated compound as Pentachlorophenol (PCP).

P. chrysosporium (Reddy and Gold, 2000*)* and *T. V*ersicolor (Sedarati *et al*., 2003) have been the most widely used fungi for PCP degradation. Nevertheless, diverse studies have been performed to evaluate new fungal strains with a high ability for the degradation of recalcitrant organic compounds like chlorophenols (Levin *et al*., 2004; Rubilar *et al*., 2007, Tortella *et al*., 2008). Several fungi, such as *Agrocybe semiorbicularis*, *Auricularia auricula*, *Coriolus versicolor*, *Dichomitus squalens*, *Flammulina velupites*, *Hypholoma fasciculare*, *Pleurotus ostreatus*, *Stereum hirsutum,* and *A. discolor,* have shown their ability to degrade various pesticide groups like phenylamide, triazine, phenylurea, dicarboximide, chlorinated and organophosphorus compounds (Bending *et al*., 2002). *P. chysosporium* degraded isoproturon belonging to phenylurea groups (Wirén-Lehr *et al*., 2001), *B. adusta* and *A. discolor* Sp4 the pesticide pentachlorophenol (Rubilar *et al*., 2007), and *T. versicolor* and *Agaricus augustus* degraded 2,4,6-tribromophenol (Donoso *et al*., 2008).

Biodegradation of pentachlorophenol in soil slurry cultures by *B. adusta* and *A. discolor* Sp4 was studied by Rubilar *et al*., 2007. The high degradation rates obtained during the fungal cultures were due to the agitation imposed to maintain the slurry phase, which, in turn, increased the bioavailability of the pollutant to the fungi. Between the two fungal strains, *A. discolor* Sp4 presented the best results in terms of ligninolytic activity and PCP

degradation potential. These parameters were increased when a lignocellulosic waste was used as a substrate. However, no correlation between PCP degradation and ligninolytic activity was observed either from the small flask cultures or from the soil slurry system in the stirred tank reactor. The degradation pathway of pentachlorophenol by *A. discolor* Sp4 in the soil slurry reactor has been established by Rubilar (2007) and is shown in Figure 2.
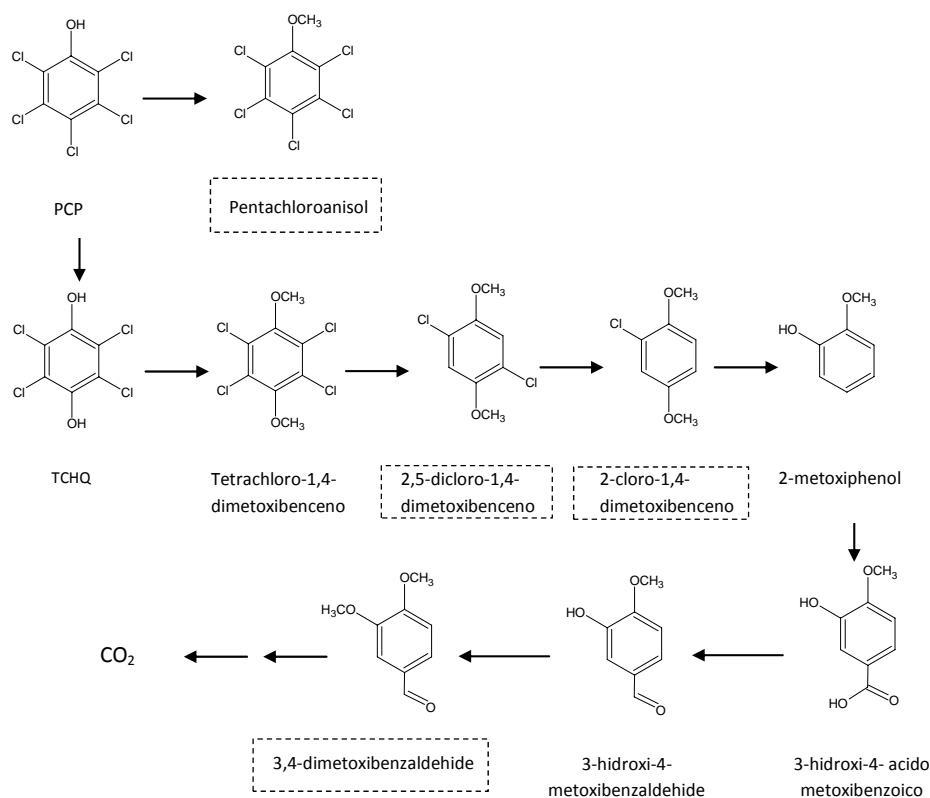
Figure 2. Proposed pathways for the degradation of pentachlorophenol by *Anthracophyllum discolor* in soil slurry reactor. Framed compounds correspond to those identified by GC-MS (Rubilar, 2007).

The enzymatic characterization of Chilean native wood-rotting fungi for their potential use in the bioremediation of environments polluted with chlorophenols has been studied by Tortella *et al*. (2008). Eleven strains were selected and showed high concentrations of lignin peroxidase (Lip) and manganese peroxidase (MnP).

A. discolor (Sp4) produced LiP and MnP at 90.3 and 125.5 U L^{-1} respectively, compared with the control fungus *P. chrysosporium* CECT-2798 that produced 58.1 and 118.4 U L^{-1} of LiP and MnP, respectively. Tolerance tests showed that native Chilean fungi did not present high tolerance to 2,4,6-TCP and PCP, but they

were quite tolerant to 25 and 50 mg L^{-1} of 2,4-DCP. However, pre-acclimatization in 2,4-DCP notably improved the growth in medium with 2,4,6-TCP and PCP. PCP in liquid medium was efficiently degraded by the fungi *A. discolor*, *Lenzites betulina* (Ru-30) and *Galerina patagónica* (Sp3), and the highest MnP activity was produced by *A. discolor* (Sp4) (67 U L^{-1}).

TECHNOLOGIES FOR PESTICIDE MITIGATION IN THE ENVIRONMENT

Several different technologies available for the treatment of pesticide-contaminated sites have been reported (Wait and Thomas, 2003; Aitken and Long, 2004; Chaudhry *et al.* 2005; De-Wilde *et al.*, 2007; Shaalan *et al.*, 2007). These are based on physical, chemical and biological treatments. Ideally, a treatment will result in the destruction of the compound without the generation of intermediates. Some technologies are only capable of relocating or stabilizing a contaminant and do not result in its destruction. In general, it has been observed that these treatments present high removal efficiency; nevertheless, they present diverse costs and variable times of treatment for the removal of these pollutants. No single specific technology may be considered as a panacea for all contaminated site problems. A complete analysis of the site restoration techniques that may be employed in a variety of contaminated site cleanup programs has been reviewed by Khan *et al.* (2004). An easy-to-use summary of the analysis of the important parameters that will help in the selection and implementation of one or more appropriate technologies in a defined set of sites and contaminant characteristics is also included in the review of Khan *et al.* (2004).

Bioremediation process can be divided into three phases or levels. First, through natural attenuation, contaminants are reduced by native microorganisms without any human augmentation. Second, biostimulation is employed where nutrients and oxygen are applied to the systems to improve their effectiveness and to accelerate biodegradation. Finally, during bioaugmentation, microorganisms are added to the systems. These supplemental organisms should be more efficient than native flora to degrade the target contaminant (Salinas-Martínez *et al.*, 2008). Inside this context, several methodologies for removal of toxic compounds in wastewater through biodegradation process have been evaluated, among some the use of slurry batch bioreactor with soil (Quintero *et al.*, 2007; Rubilar *et al*, 2007), organic waste addition (Aslan and Türkman, 2005; Cea *et al.*, 2007b; Trejo-Hernández *et al.*, 2007; Seo *et al.*, 2007), soil-organic waste mixture (Vischetti *et al.*, 2004), and microorganisms inoculation in organic supports (Bending *et al.*, 2002; Quintero *et al.*, 2007; Wu and Yu, 2008).

The current knowledge on mitigation strategies to reduce pesticide inputs into surface water and groundwater, and their effectiveness when applied in practice has been reviewed by Reichenberger *et al.* (2007). In the review, the mitigation measures identified in the literature are evaluated with respect to their practicability, and those measures considered both effective and feasible are recommended for implementing at the farm and catchment scale. On the other hand, Castillo *et al.* (2008) have presented the state of the art of biobeds, a low-cost technology for point source contamination for pesticide mitigation, and similar systems in Sweden and worldwide and identified future research needs. Factors affecting the efficiency of biobeds in

terms of degradation and retention of pesticides are discussed, with particular emphasis on the microbial processes involved.

**Pesticides degradation in biobeds**

Biobeds are a biological system originated in Sweden as a response to the need for a simple and effective system to minimize environmental contamination from pesticide manipulation, especially when filling the spraying equipment, a typical point source of contamination (Figure 3) (Tortensson and Castillo, 1997; Castillo *et al*., 2008). In Sweden and in other countries of Europe, great quantities of biobeds are functioning in farms and they have shown to be efficient at reducing pesticide water-body contamination (Castillo *et al*., 2008; Vischetti *et al*., 2007). Biobeds are based on the adsorption and degradation potential of organic biomixtures composed of top soil, peat, and straw and covered with grass (Tortensson and Castillo, 1997). Biobeds are a low-cost alternative for treating pesticide waste and washings, providing a matrix to absorb the pesticides and facilitate their biodegradation. Straw stimulates growth of ligninolytic microorganisms and the production of extracellular ligninolytic enzymes as peroxidases and phenoloxidases. The peat contributes to sorption capacity, moisture control and also abiotic degradation of pesticides and also decreases pH of the biomixture, which is favorable for fungi and their pesticide-degrading enzymes (Castillo *et al*., 2001; Torstensson and Castillo, 1997). Soil enhances the sorption capacity in the biobed. It should have humus and clay content to promote microbial activity. Moreover, it is also an important source of pesticide-degrading bacteria or Actinomycetes that can act synergistically with the fungi. Chilean Andisol is an effective support for this system due to its high organic matter content with great affinity for pollutants due to the presence of humic and fulvic acids and reactive clays like Al and Fe hydroxide groups (Navia *et al*., 2005).

Several studies have demonstrated that biobeds can effectively retain and degrade pesticides (Torstensson and Castillo, 1997; Castillo *et al*., 2001; Fogg *et al*., 2003 a,b; Fogg *et al*., 2004 a,b; Vischetti *et al*., 2004; Castillo and Torstensson, 2007; Vischetti *et al.*, 2008).

Studies with mecoprop and isoproturon have shown than these pesticides can be degraded in biobeds (Henriksen *et al*., 2003). Niels *et al*. (2006) evaluated the degradation and leaching of 21 pesticides. They determined than no traces of 10 out of 21 applied pesticides were detected in the percolate. Fogg *et al*. (2003a) evaluated the ability of biobeds to degrade pesticide mixtures (isoproturon and chlorothalonil) and the concentration effect. They found that with the exception of isoproturon at concentration above 11 mg kg^{-1}, degradation was quicker in biomix than in topsoil. The degradation of either isoproturon or chlorothalonil was unaffected by the presence of the other pesticide. Fogg *et al*. (2003b) determined if biobeds can degrade complex pesticides mixtures when applied repeatedly. They reported that degradation was significantly quicker in biomix than in topsoil; however, it decreased with each additional treatment. Fogg *et al*. (2004a) studied the degradation and leaching potential of pesticides at the semi-field scale, in a lined biobed and unlined biobed system. They reported that only the most mobile pesticides (K_{oc} <100) leached when unlined biobed was used, and for these >99% was removed by the system, with a significant proportion degraded within 9 months.

Laboratory-based studies showed that mixtures of soil-organic waste may be able to degrade high concentrations and complex mixtures of pesticides (Fogg *et*
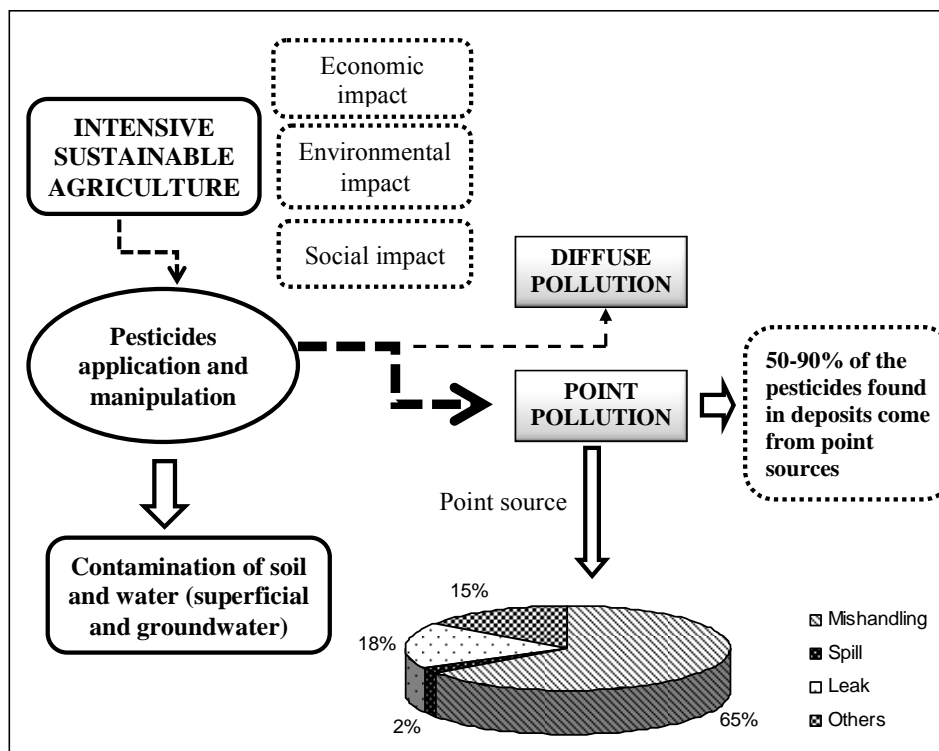
Figura 3. Diagram of effects of contamination associated with pesticides application and manipulation and, distribution (%) of the point source contamination during handling.

al., 2004b). However, factors such as composition, flow, concentration of influent and bed depth are crucial parameters for the fixed bed column operation (Wu and Yu, 2008). Consequently it is important to establish the proportion of materials in the column mixture giving the highest degradation and sorption efficiency. Castillo and Tortensson, (2007) observed that a straw: peat: soil ratio of 50:25:25% v/v is recommended for the organic mixture composition, because such mixture favors a low pH, convenient for lignin-degrading fungi and phenoloxidase production and activity.

Vischetti *et al.* (2004) compared the behavior of chlorpyrifos in two biobed systems: a Swedish biobed and a modified Italian system of biobeds. They reported that chlorpyrifos half-lives were similar in both evaluated biomixtures, but the microbial biomass content was reduced by 25 and 50% with 10 and 50 mg kg^{-1} of chlorpyrifos in the Italian biomix, respectively. Coppola *et al.* (2007) and Vischetti *et al.* (2007) studied the biodegradation of chlorpyrifos in biobeds system adapted to Italian conditions. They found that the Italian biomix showed several differences, compared with

Swedish biomix in the chlorpyrifos degradation.

The effect of initial concentration, co-application and repeated applications on chlorpyrifos and metalaxyl degradation were studied in a biobed mixture by Vischetti *et al*. (2008). The authors concluded that biomix in biobed degraded both pesticides relatively fast and had a microbial community that is varied enough to allow selection of those microorganisms able to degrade both pesticides.

Several pesticides were degraded in biobeds inoculated with white-rot fungi (Bending *et al*., 2002). The authors determined that some pesticides were degraded by about 86% in biobeds; however, pesticides as chlorpyrifos and terbuthylazine were less degraded. Wirén-Lehr *et al*. (2001) studied the degradation of isoproturon in biobeds with and without inoculation with white-rot fungus *Phanerochaete chrysosporium*. They determined that a strong decrease in the concentration of isoproturon was observed in non-inoculated biobeds. Total extractable isoproturon decreased by 76% after 100 days. However, the decrease was even larger in biobeds inoculated with the white rot fungus *P. chrysosporium*. After 28 days, total extractable isoproturon decreased by 78%, and after 100 days >99 % had disappeared in the biobeds.

Although, it has been shown that biobeds can be used to degrade pesticides, there is no information in the literature about their improvement through strategies such as biostimulation.

Tortella, *et al*. (2009) studied whether biomix of biobed systems with allophanic top soil (Andisol) from southern Chile can degrade effectively the organo-phosphorous insecticide chlorpyrifos. The results of this study showed that pre-incubation time of biomix with allophanic soil between 15-30 days had no major effect on the chlorpyrifos degradation. High chlorpyrifos concentration can be degraded in biobed, and the metabolite 3,5,6-trichloro-2-pyridinol (TCP) was formed, but it also can be degraded by this system. Pentachlorophenol (PCP) degradation in two biological systems (biobed and fixed-bed column) has been studied by Diez and Tortella (2008). Both systems were inoculated with *A. discolor* Sp4 immobilized in lignocellulosic material. In the biobed system, degradation of PCP (after three successive applications of 100 mg kg^{-1} each) occurred efficiently over the whole incubation period. However, it was significantly quicker in biobed amended with the fungus compared with non-inoculated biobed. A fraction of the initial PCP concentration was adsorbed (aprox. 40%) in each application by the biobed matrix. The manganese peroxidase activity was the highest in inoculated biobeds, and the laccase activity was the highest in control biobed. In fixed-bed column, PCP adsorption onto the soil was high in the initial step (12 h) and then PCP was efficiently degraded until saturation of the columns (day 16). The highest degradation of PCP coincides with the MnP production, showing that *A. discolor* Sp4 increased degradation of PCP.

CONCLUDING REMARKS

Some of the synthetic chemicals are extremely resistant to biodegradation by native flora compared with the naturally occurring organic compounds that are readily degraded upon introduction into the environment. The dissipation of organic recalcitrant pollutants in soil environment is influenced by both adsorption and biological processes. Depending on the soil properties, the biological decomposition of pesticides is the most important and effective way to remove these compounds from this

environment. White-rot fungi can degrade pesticides effectively with advantages associated with their ligninolytic enzymatic system. These fungi play an important role in the dissipation of pesticides in biobeds, an effective system to minimize environmental contamination from pesticide manipulation. However, further studies are needed to evaluate alternatives of bioaugmentation of biobed systems with inoculum of native fungi or consortium of fungi and Actinomycetes, as well as new technologies to protect these inocula of autochthonous microflora depletion must be developed.

ACKNOWLEDGEMENTS

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