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Biodegradation and Bioremediation of Organic Pesticides

Jesús Bernardino Velázquez-Fernández, Abril Bernardette Martínez-Rizo, Maricela Ramírez-Sandoval and Delia Domínguez-Ojeda

Additional information is available at the end of the chapter

1. Introduction

Pesticides can be used to control or to manage pest populations at a tolerable level. The suffix "-cide" literally means "kill", therefore, the term pesticide refers to a chemical substance that kills pests. It is incorrect to assume that the term pesticide refers only to insecticides. Pesticides include many different types of products with different functions or target (Table 1). The pesticide designation is formed by combining the name of the pest (e.g., insect or mite) with the suffix "-cide" (1).

Pesticides could be classified according to their toxicity, chemical group, environmental persistence, target organism, or other features. According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 persistent organic chemicals are pesticides. Classes of organic pesticides (consisting of organic molecules) include organochlorine, organophosphate, organometallic, pyrethroids, and carbamates among others (2, 3).

Most pesticides cause adverse effects when reaching organisms. The intensity of the toxic effect varies with time, dose, organism characteristics, environmental presence or pesticide characteristics. Their presence in environment determines the dose and time at which an organism is exposed and could represent a hazard for worldwide life due to their mobility. Hence, the persistence in the environment leads to a risk for life: the more persistent a pesticide is, the worse its environmental impact.

Pesticide persistence in environment is caused by either their physico-chemical properties or the lack of organisms able to degrade them. Light, heat or humidity could lead to loss of some pesticides by either volatilization or degradation (4). Contrastingly, degradation caused by organisms (biodegradation) could help decreasing considerably the pesticides persistence in environment. This information could be used to improve elimination of the

undesirable effects of pollutants by using organisms; such an approach has been called bioremedation.

The ability of organisms to bioremediate pesticides is mainly based on their biodegradation activity. Though bioremediation has been firstly achieved using microorganisms (bacteria or fungi), other organisms like plants or algae can be used. The aim of the present paper is to review the metabolic features which make organisms useful for bioremediation.

2. Overview

At this point, it is worth to mention that there is no convention on some words used in biodegradation. Here, we propose some words to improve communication and understanding bioremediation strategies. Albeit discussion of proper words is beyond aim of the present paper, we believe that before continuing is important to set up some concepts.

"Bioremediation" refers to any strategy used to eliminate undesirable effects of pollutants from environment. It would be desirable to eliminate pollutants but this is not always possible; though, some organisms could confine or immobilize them. For instance, organisms can accumulate contaminants, and reduce their presence and their environmental effect, but do not eliminate them from the environment. Such strategy, which is actually used (v.gr. phytoextraction (5)) should be included into the "bioremediation" concept. Those organisms able to bioremediate would be called bioremediators.

Pesticide	Target
Algicides	Algae
Avicides	Birds
Bactericides	Bacteria
Fungicides	Fungi
Insecticides	Insects
Miticides or Acaricides	Mites
Molluscicides	Snails
Nematicides	Nematodes
Rodenticides	Rodents
Virucides	Viruses

Table 1. Classification of pesticides according to their target.

Traditionally, bioremediation has been achieved by using microorganisms. Nevertheless, The fact that in past decades, several reports on bioremediation using plants, fungi, algae or enzymes (obtained from organisms) has broadened the scope of bioremediation. Words like phytoremediation or rhizoremediation have been used (5, 6), and perhaps it would be necessary to name properly each bioremediation strategy regarding the organism used (Table 2).

Bioremediator organism	Strategy
Microorganism	Microbioremediation or Bioremediation
Bacteria	Bacterial bioremediation
Fungi	Mycoremediation
Plants	Phytoremediation
Rhizosphere	Rhizoremediation
Algae	Phycoremediation
Biomolecules derived from organisms	Derivative bioremediation

Table 2. Classification of bioremediation strategies according to the organism involved.

The concepts of biodegradation and biotransformation overlap extensively, so that, they are synonymous in appearance. Biodegradation involves the biological reactions that modify the chemical structure of the compound, so, this implies a decrease in toxicity. In contrast, biotransformation reduces the pollutant concentration by either modification or translocation. Thus, biotransformation could end decreasing or increasing the undesirable effects. Their difference is clear in the case of pollutants translocation when biodegradation is not occurring but biotransformation does. Biotransformation concept has been developed for biological detoxification systems (7) and is a key concept in bioremediation strategies because they both are intended to eliminate undesirable effects of pollutants to organisms. Along the text, the word "Biodegrader" will be used for the organism able to biodegrade a certain compound. "Mineralization" refers to biodegradation leading to compounds like CO_2 or NH_3 which could be biologically assimilated (8).

In the earliest works on bioremediation, the practical purpose was to find or to isolate biodegrader microorganisms or consortia. In an admirable work, Alexander (8) reviewed several biodegrader consortia found in polluted environmental matrixes (soil, sediment or water). Among those tolerant or adapted microorganisms, there might be some proper bioremediators. A plausible explanation for this phenomenon might be that pesticides have exerted evolutionary pressure, so that, only organisms able to tolerate those doses of pesticides will survive. Even though not every tolerant organism is a biodegrader, every biodegrader should be tolerant. Thus, the evolutionary pressure exerted by the pollutant would have selected some tolerant bioremediators. In keeping with this, traditionally, bioremediation studies measured only final concentration of pollutants, but little or no attention to biochemical mechanisms responsible for biodegradation was given. Further research on factors affecting biodegradation process is required to improve selection of bioremediators and application of bioremediation technologies.

2.1. Factors affecting biodegradation process

Some metabolic features related to biodegradation efficiency have been investigated for microorganisms (8). Any factor which can alter growth or metabolism, would also affect biodegradation. Hence, physicochemical characteristics of the environmental matrix, such as temperature, pH, water potential, oxygen and substrate availability, would influence the

biodegradation efficiency (Figure 1). Two more factors are worth to mention: co-metabolism and consortia condition. Some biodegraders need other substrates to degrade pollutants (8). This phenomenon is called co-metabolism and is especially required for organochlorine compounds. In contrast, it has been shown that the presence of other carbon sources decreases organophosphate biodegradation (9).

When pesticide degradation occurs, it usually involves more than one microorganism, i.e. each microorganism contributes to biodegradation reactions on pesticides, but no example of mineralization by a single strain has been described. It seems that the presence of different microorganisms is essential for an adequate biodegradation. Reported microbiodegraders belong to basidiomycetes or to bacterial classes: gamma-proteobacteria (v.gr.: *Pseudomonas*, *Aerobacter*, *Acinetobacter*, *Moraxella*, *Plesiomonas*), beta-proteobacteria (v.gr.: *Burkholderia*, *Neisseria*), alpha-proteobacteria (v.gr.: *Sphingomonas*), actinobacteria (Micrococcus) and flavobacteria (*Flavobacterium*).

Pollutants might undergo biodegradation reactions like de-chlorination, cleavage, oxidation, reduction by different enzymes. Since biodegradation ability is based on enzymes which are promiscuous and have evolved to detoxifying enzymes, the shorter the duplication time of organism, the more adequate the organism is and the easier to obtain biodegraders. Thus, bacteria with duplication time around minutes are likeable to respond to natural or artificial pollutant-induced evolutionary pressure; this response consists in selecting biotransformation enzymes able to degrade them. These promiscuous enzymes are present in organisms even before the exertion of the evolutionary pressure, which could have induced genetic recombination or mutation leading to enzymes with better biodegradation ability. Copley (10) has excellently reviewed the evolution of metabolic pathways and those factors affecting the efficiency of pollutant biodegradation.

Though bacteria have been proved to be good biodegraders and bioremediators, some fungi, plants and algae could biodegrade pesticides too. Knowing the metabolism of those biodegrader species or strain improves the selection of bioremediation strategy for each site either by biostimulating the indigenous biodegraders (biostimulation) or adding exogenous to the site (bioaugmentation). Moreover, thanks to molecular biology, the metabolic biodegradation ability could be transferred from a biodegrader to another organism, thus improving its degrading capabilities. For instance, using genetic engineering, a whole mineralization pathway for paraoxon –the oxon metabolite of the organophosphate pesticide parathion- was built in a single strain of *Pseudomona putida* (11). Taking all this into account, it is clear that biodegradation enzymes play a key role in bioremediation processes and their knowledge could help in designing or choosing the most adequate strategy.

Biotransformation enzymes have been traditionally classified according to the phase they participate. There are three phases of biotransformation. Phase I consists of those enzymes catalyzing reactions which modify pollutant functional groups. In phase II, those enzymes catalyzing transfer reaction of whole groups or biomolecules to pollutants are classified. Phase III includes translocation processes rendering pollutants or their metabolites non bioavailable. For bioremediation purposes, biotransformation enzymes mainly belong to

four biochemical types: oxidoreductases, hydrolases, transferases and translocases (or pumps). Among oxidoreductases, the most frequent are monooxygenases (like cytochrome P450), dioxygenases, peroxidases and oxidases. Hydrolases like A-esterases are involved in biodegradation pathways. There are many types of transferases, and they are classified according to the group they conjugate to the xenobiotic: methyl-transferases, acetyl-transferases, glutathione S-transferases among others. For bioremediation purposes, only a couple of translocases have been identified and characterized: boths are pumps that translocate herbicides or glutathione-conjugates to vacuoles.
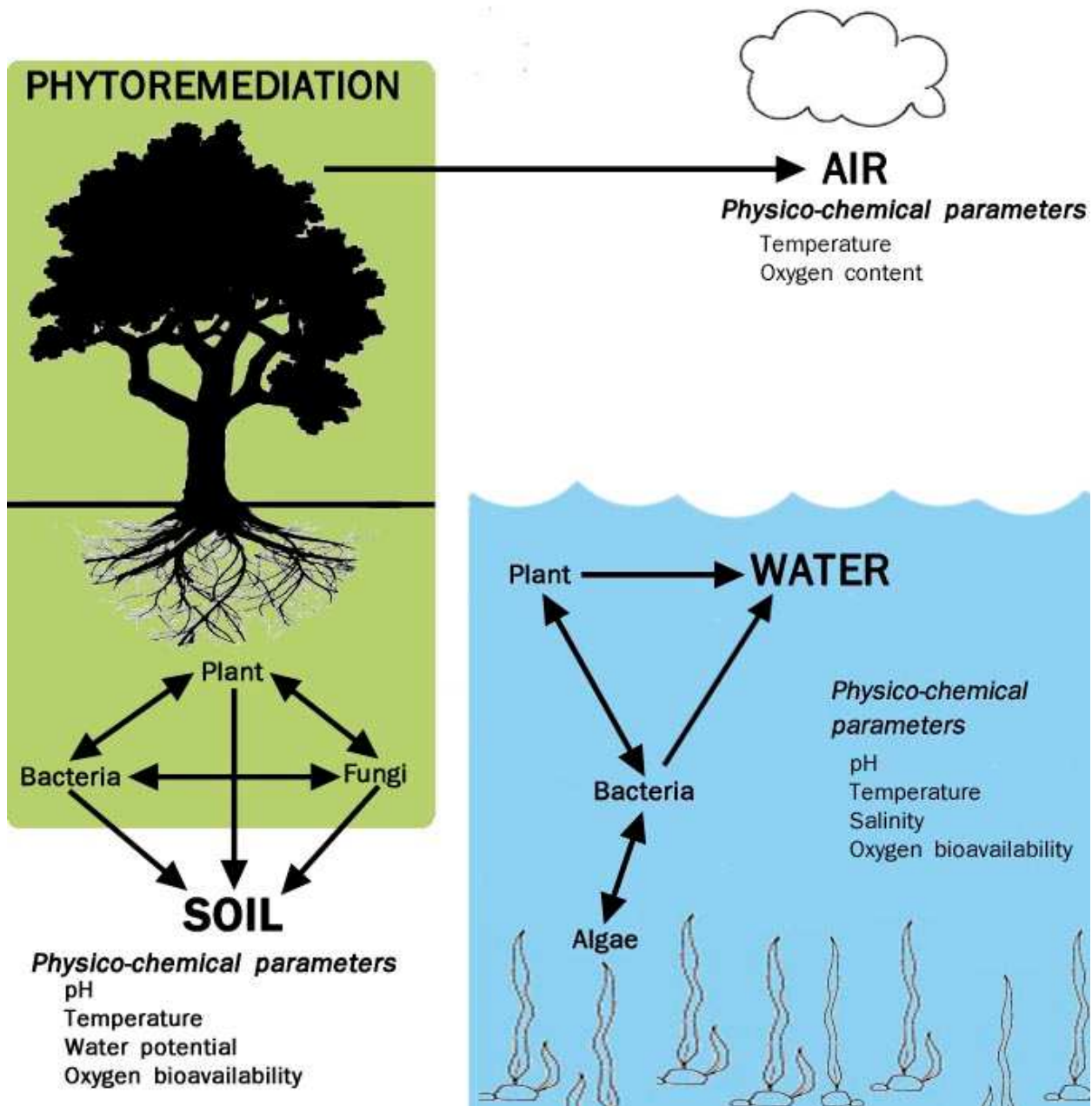
PHYTOREMEDIATION

AIR
Physico-chemical parameters
Temperature
Oxygen content

Plant ⟶ WATER

Plant

Bacteria ⟷ Fungi

Physico-chemical parameters
pH
Temperature
Salinity
Oxygen bioavailability

Bacteria

SOIL

Algae

Physico-chemical parameters
pH
Temperature
Water potential
Oxygen bioavailability

Figure 1. Factors affecting biodegradation and bioremediation in soil, water or air.

The biotransformation of every pollutant could be catalyzed by different enzymes depending on organism. There is no a sequence of reaction pre-determined and is independent of the classification described above. Detoxifying enzymes are promiscous and have different affinities and velocities. Their protein nature makes them susceptible to different factors like heat, pH or substrate availability. In general, biotransformation enzymes for bioremediation are present in bacteria, fungi, plants and animals. In the next section, main enzymes from bacteria, fungi and plants involved in organic pesticide degradation are briefly described. Afterwards, some examples of bacterial, plant, fungi or algae bioremediators are reviewed.

Cytochrome P450 (CYP): This consists of a superfamily of heme monooxygenases. They can catalyze reactions of oxidation, reduction or oxidative breakdown of xenobiotics (Figure 2). It seems that they are evolutively conserved since genomes from virus, bacteria, algae, plant, fungi and animals have isoforms of CYP codified (12-21). In eukaryotic organisms, CYP is found in smooth endoplasmic reticulum, and can biotransform a wide range of pollutants. A review about the biology of CYP can be found elsewhere (22). CYP catalyzes biodegradation of aromatic or alyciclic compounds and can activate toxics, i.e., CYP action on biomolecules might make them toxic or increase their toxicity.
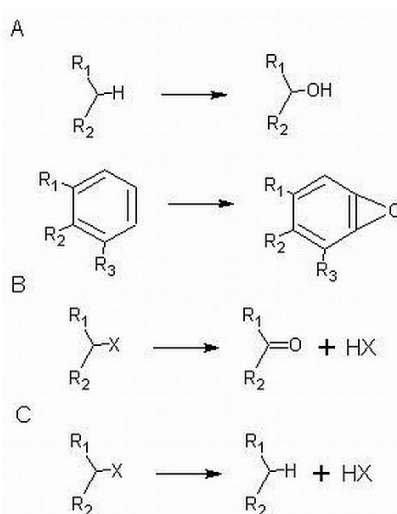

Figure 2. Scheme of reactions catalyzed by CYP: A) oxidation (monooxygenation), B) oxidative and C) reductive dehalogenation.

A-esterases: Esterases can be classified according to their interaction with organophosphates. A-esterases can catalyze the hydrolysis of organophosphate or carbamate pesticides (Figure 3), B-esterases are inhibited by organophosphates and C-esterases show no interaction with organophosphates. A-esterases include several enzymes like monophosphatases, phosphodiesterases or phosphotriesterases. They frequently use calcium and have been found in bacteria, fungi and animals (23). Human paraoxonase is an A-esterase and is involved in susceptibility to organophosphate pesticides; a review on human PON1 could be found elsewhere (24).
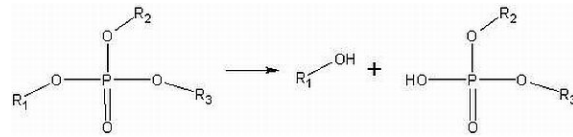
Figure 3. Scheme of reactions catalyzed by A-esterases.

Peroxidases and oxidases: They include some families of enzymes catalyzing redox reactions (Figure 4). Although they are produced by bacteria, fungi, plants and animals, reports on pesticide biodegradation exist for fungi. Peroxidases participate in cell response to oxidative damage and most of them are metalloproteins. They are extemely sensitive to the presence of azide, and inhibitor of metalloenzymes, with the exception of lignine peroxidases from fungi (25). It is known that ligninolytic fungi secrete peroxidases and oxidases to degrade lignine (25, 26). These enzymes are highly promiscuous.
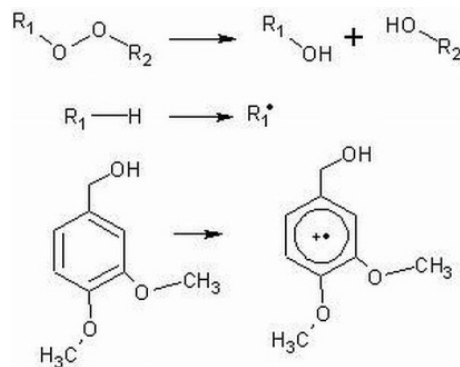

Figure 4. Scheme of reactions catalyzed by peroxidases.

Transferases: Among all known transferases, Glutathione S-transferase (GST) is the mainly involved in biodegradation for bioremediation purposes. GST includes a superfamily of enzymes that have been found in bacteria, fungi, algae, plants and animals (27-29). Even though they catalyze transference of glutathione to electrophillic pesticides, they can also show hydrolytic and peroxidase activities (29). Interestingly, GST can also catalyze the de-halogenation of rings (Figure 5, (30)).
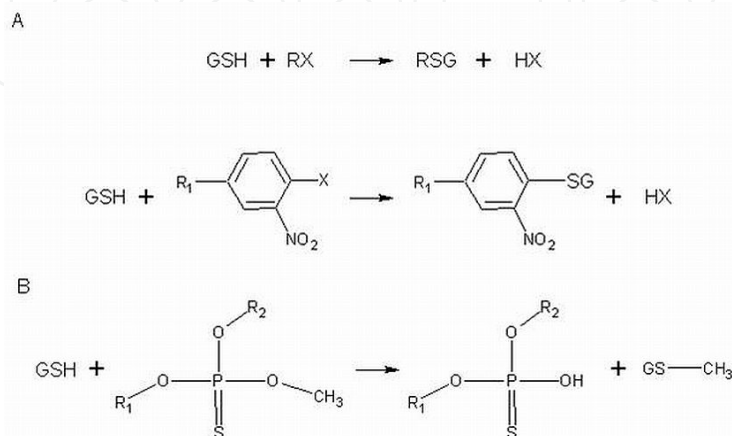

Figure 5. Scheme of reactions catalyzed by GST: A) dehalogenation, B) O-dealkylation.

Translocases: Translocation of molecules from a cell compartment to another is catalyzed by pumps named translocases. Some translocases are involved in the bacterial resistance to drugs, but this activity seems to lack relevance for bioremediation. Although it does not constitute a biodegradation itself, translocation is perhaps the only step of phase III biotransformation. In plants, translocation is part of secondary metabolism and herbicide-tolerance; interestingly, it has been suggested that a previous glutathionation is required for translocation to vacuoles (31, 32).

3. Bacterial bioremediators

Bacteria have been used extensively for bioremediation purposes. These studies have focused on the employment of bacteria, consortia or on the search for biotransformation enzymes. The fast growth, easy handling and low cost make them suitable for bioremediation. Unfortunately, there are some disadvantages such as the disposal of bacterial biomass, pathogenicity, bioactivation, among others. Bacteria can be found in soil, water or even in particles dispersed in air. Unfortunately, only a small fraction of bacteria (<10% from soil) can be cultured in laboratory conditions (33). Because of this, the number of studies about pesticide biodegradation mechanisms is less than those about biodegraders isolation, and then, little information on biochemical mechanisms or enzymes is available. For organochlorine pesticides, only few biodegration enzymes and genes have been described.

Bacterial biodegradation could take place in anaerobic or aerobic conditions. Although different enzymes participate in each condition, it seems that both, aerobic and anaerobic degradation should happen if a mineralization is expected to occur (34). It seems that anaerobic metabolism is more adequate for dechlorination (35, 36) and aerobic metabolism produces a cleavage in aromatic or aliphatic cyclic metabolites. The higher persistence of organochlorine in aerobic conditions (37) compared to anaerobic might be caused by the absence of enzymes or more likely by the oxidative damage following organochlorine metabolism. The removal of heteroatoms (like halogens) or heteroatom-containing groups are frequently among the first steps in biodegradation. These steps are catalyzed by monooxygenases, dioxygenases or peroxidases (37, 38), which in aerobic conditions could generate large quantities of free radicals. Thus, anaerobic conditions are more adequate for biodegradation of organochlorine pesticides, while aerobic are better for biodegrading hydrocarbon metabolites from pesticides (5). In spite of such requirements, some examples of organochlorine pesticides bioremediation could be accomplished *in situ* (34, 39).

Baczynski and co-workers(36) demonstrated that anaerobic biodegradation of dichlorodiphenyltrichloroetano (DDT), metoxychlor and gamma-hexachlorociclohexane (gamma-HCH), is affected by temperature and the ratio of desorbed pesticide. Moreover, only on chlorine atom could be cleaved from DDT in those conditions. This is in agreement with that reported by Alexander (8) who pointed out that biodegradation could produce molecules with at least one chlorine atom. Bacteria related to *Pseudomonas*, *Neisseria*, *Moraxella* and *Acinetobacter* able to degrade almost completely DDT were isolated from Yaqui valley in Sonora, Mexico (40). However, no information on biodegradation mechanism was compiled out.

Anabaena (a cyanobacterium), *Pseudomonas spinosa*, *Pseudomonas aeruginosa* and *Burkholderia* were shown to be good biodegraders of endosulfan (41, 42). The biodegrader KS-2P strain of *Pseudomona* was isolated from endosulfan polluted soil by repetitive enrichment in cultures. This strain could reduce the endosulfan concentration in days in a dose-dependent manner. As far as we know, no mineralization of endosulfan has been observed. Microorganisms from the *Pseudomonas*, *Bacillus*, *Trichoderma*, *Aerobacter*, *Muchor*, *Micrococcus* and *Burkholderia* genera have been shown to biodegrade dieldrin and endrin (43).

Even when HCH is considered as a persistent organic pollutant, it has been demonstrated that it could be bioremediated in situ (34). Murthy and Manonmani (44) identified a HCH-biodegrader consortium which contained species from *Pseudomonas*, *Burkholderia*, *Flavobacterium* and *Vibrio* genera. The biodegradation was achieved within hours. An excellent review by Phillips and co-workers (45) describes and enlists several HCH biodegraders. Interestingly, they could be grouped in two bacteria (*Sphingomonas* and *Pseudomonas*) and one white rot fungi (*Phanerochaete chrysosporium*). HCH mineralization seems to need aerobic and anaerobic conditions like those provided by particles, i.e. in one hand, oxygen could be bioavailable in soil, on the other, soil particles may present niches for anaerobic metabolism. This could explain also why bacteria grown on coffee beans exhibit better biodegradation than those in medium alone (35). Genes encoding enzymes able to degrade gamma-HCH have been named *lin* (37, 46), but further research on biochemical characterization is needed. Comparing biodegradation times for HCH, DDT and endosulfan, differences are observed. Listed in an increasing order of needed time for biodegradation: HCH<DDT<endosulfan. Evidently, this time varies according to the consortium or strains used.

It has been shown that some bacteria could degrade parathion (47) and fenitothrion by using A-esterases (48). From soil, Singh et al. have isolated a strain related to *Enterobacter* which can mineralize chlorpyrifos, parathion, diazinon, coumaphos and isazofos (49). Similarly, it has been found that a bacterial biodegrader related to *Serratia* can degrade diazinon (50). The A-esterase, can be encoded on genome or plasmid. A gene from the genome of a strain related to *Plesiomonas* which can hydrolize methylparathion was cloned to *Escherichia coli* (51). In contrast, the ability to degrade fenitothrion by a *Burkholderia* strain was found to be encoded on plasmids (9). Unfortunately, the presence of other carbon or phosphorous sources reduces the efficiency of organophosphate biodegradation. This limits severely the application of these biodegraders on bioremediation. Further research about parameters influencing biodegradation efficiency is needed to improve their usefullness for bioremediation.

4. Phytoremediators

Phytoremediation –the use of plants for bioremediation- has been less studied than those strategies using bacteria. Nevertheless, it has been proved to be more effective at large scale for soil, water and even for air pollution than bacteria. The mechanisms involved in the phytoremediation success include several bioremediation strategies like phytoextraction,

rhizodegradation, rhizofiltration, phytodegradation, phytostabilization (5, 52) (Figure 6). Several factors affect phytoremediation efficiency (Table 3). The enzymes involved in plant biotransformation are mainly CYP, carboxylesterases, GST and translocases (52). When using a plant, some cautions have to be considered; for instance, introduction of new species should be avoided and plant should tolerate transplantation and pesticide exposition. (5). Ramírez-Sandoval et al., (53) have showed that transplantation itself could induce oxidative stress in plant itself.

Phytodegradation and phytoextraction are the key mechanisms of plant defense (54). Maize (*Zea mays*) and giant foxtail (*Setaria faberi*) can biotransform some herbicides (55). Crop plants like brinjal (*Solanum melongena*), spinach (*Spinacea oleracea*), radish (*Raphanus sativus*) and rice (*Oryza sativa*) can bioaccumulate pesticides like DDT and benzene hexachloride (56). Basil (*Ocimum basilicum*) can bioremediate endosulfan from soil (53). Barley (*Hordeum vulgare*) can translocate herbicide metolachlor into vacuoles (31). Horseweed (*Conyza canadensis*) sequesters glyphosate in vacuoles (57). Also, it has been suggested that genetic engineering could be used to improve phytoremediation abilities of poplars (58) and plants in general (59).
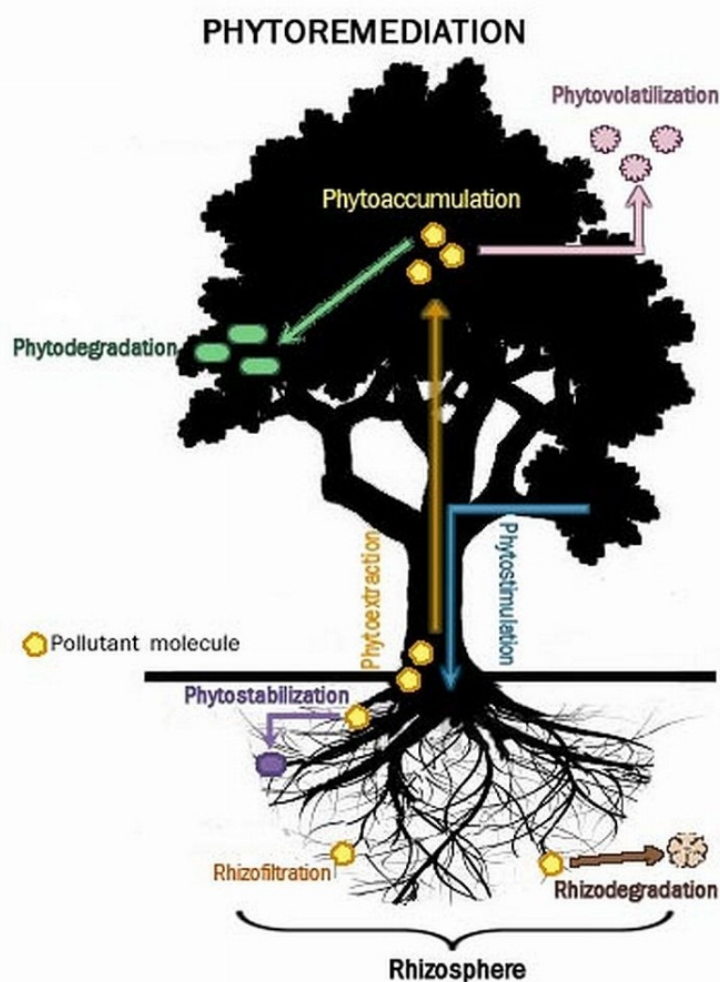

Figure 6. Mechanisms concerning in phytoremediation.

| Absorption | Elimination | |
	Physical	Chemical
Site of the plant	Roots, leaves	Leaves, vacuoles	Rhizosphere, plant
Mechanism	Phytoextraction, Rhizofiltration	Phytovolatilization, Phytoaccumulation	Rhizodegradation, phytostimulation, phytodegradation
Limiting factors	Temperature, pH, molecular weight, hydrophobicity	Pollutant concentration, plant defense mechanisms	Class of pollutant, enzymes presence, bioavailability

Table 3. Factors involved in phytoremediation.

Since biodegrader microorganisms can be found in rhizosphere, pairs of plant-rhizosphere are unequivocally better bioremediators than taken separately. Plants exudate carbohydrates and mucilages that stabilize and nurture microorganisms around roots, providing better conditions for microbial growth than soil alone. As a matter of fact, the amount of microorganims around the plants roots are 10- to 100-fold those found in soil alone (60). In addition, some plants can provide co-substrates and oxygen to rhizosphere microorganisms, stimulating them to biodegrade pesticides. Phytostimulation has proved to be one of the most helpful strategies since it brings together the bioremediation capabilities of plant and its biorizhosphere -bacteria and mycorhiza (61).

The efficiency of the phytoremediation depends on several parameters like species, substrate, plant tolerance to pollution, among others. Nevertheless, phytoremediation has several advantages such as the control on bacterial biomass, the slow growth leading to few amounts of plant biomass, the large amounts of soil that could be treated. There are disadvantages or limitations such as the decrease in soil content needed for agriculture, times for accomplish bioremediation longer than microbioremediation, absence of native plants in the ecosystem, among others. Enzymes from microorganisms largely contribute to bioremediation when phytostimulation is performed. Because of this, some successful cases of phytoremediation could be explained by a combination of phyto- and rhizodegradation (53). Rhizoremediation have been used for remediation of the insecticide parathion and the herbicide 2,4-dichlorophenoxyacetic or 2,4-D (6). Pea (*Pisum sativum*) can stimulate endophytic bacteria to also degrade 2,4-D (62).

5. Myco- and phycoremediators

Although less studied, there is some cases worth to mention of biodegraders fungi or algae. Ligninolytic fungi have proven to be good bioremediators. Unfortunately, the nutritional, humidity and pH requirements for some species of fungi and algae represent a big obstacle for its use. Fungi secrete peroxidases, dioxygenases and oxidases able to biodegrade pesticides more efficiently than cytochrome P450 (25). Lignine peroxidase, laccase, and dichlorohydroquinone dioxygenase are some examples of biotransformation enzymes

produced by fungi like *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Ganoderma australe* and *Fusarium ventricosum*; the three former are ligninolytic, and the latter is a saprobe. *P. chrysosporium* and *F. ventricosum* are members of soil microbial community.

It has been shown that *P. chrysosporium* can biodegrade endosulfan (17); a CYP and an intracellular peroxidase are likely involved. *F. ventricosum* has been also proved to degrade endosulfan (63). It has been shown that fungal peroxidases and dioxygenases are involved in biodegradation of pentachlorophenol (64, 65). The ligninolytic fungus *Ganoderma australe*, isolated from the stone pine (*Pinus pinea*), is a good biodegrader of lindane (66). This elegant work describes several parameters which has to considered to improve biodegradation like lag time, propagation velocity, biomass growth rate, biodegradation rate, biodegradation/biomass, biomass/propagation and biomass content.

Although in less extent, there are studies on algae ability to bioremediate pesticides in water. Bioremediation appears to occur thanks to bioaccumulation and biodegradation. As in aquatic plants, the biomass overproduction could be a serious disadvantage when using algae for bioremediation waterbodies. In some species, the physicochemical water parameters and other growing conditions might be a matter of caution on choosing these organisms. The unicellular green alga *Chlorella fusca* var vacuolata is able to biotransform the herbicide Metfluorazon by a CYP (14). Recently, it has been described that the alga *Chlamydomonas reinhardtii* can bioaccumulate and biodegrade herbicide prometryne (67).

Two cases of derivative bioremediation have been reported. 1) Using minced shepherd's purse roots, herbicide 2,4-D could be successfully degraded in the presence of hydrogen or calcium peroxide. Temperature did not influence degradation and moisture increased biotransformation (68). 2) An organophosphate hydrolase was immobilized in glass. Even when the activity was decreased in 50% respect to soluble enzymes, its half-life was 280 days and its activity was independent on pH or temperature (69). It was not clear if these characteristics were derived from immobilization or was inherent to enzyme. Regardless, it is clear that immobilized enzymes could be a bioremediation alternative with some advantages, such as the avoidance of biomass production or issues with other growth requirements which have to be dealt with when working with whole organisms.

6. Advantages and disadvantages of bioremediators

Bioremediation strategies show different advantages compared to physico-chemical or thermal treatments aimed to eliminate organic pollutants from environment (Table 4). We refer to maintainable to that strategy capable of being kept from more than a year in spite of the energy, economic and human resources spent to implement it. For instance, after a pesticide release, physicochemical remediation, micro-bioremediation or phytoremediation could be used in one occasion. Nevertheless, if a continuous or an intermittent pesticide release occurs along the year, some strategies should be applied again. Microbioremediation or phytoremediation would be self-maintained through all the year, while physicochemical and some microbioremediation strategies should be implemented each time a pesticide environmental release happens.

	Physico-chemical or thermal remediation	Microbio-remediation	Phyto-remediation
Advantages/Disadvantages			
Cost	High	Low	Low
Benefit/cost ratio	Low	High	High
Environmental friendly	No	Yes	Yes
Aesthetical	No	Some cases	Yes
Self-maintainable	No	Yes	Yes
Energy expenditure	Yes	Low	Low
Uses the metabolism of several organisms	No	Some cases	Yes
Could be used to bioremediate			
Soil	Yes	Yes	Yes
Water	Yes	Yes	Yes
Air	NK	NK	Yes
Requirements			
Specific infrastructure	Yes	Some cases	No
Posterior treatments of residues	Yes	No	No
Biological control or disposal of microorganisms	NA	Yes	No
Time	Short	Medium	Large
Research on bioremediator candidates	NA	Yes	Yes
Key points			
Bioaugmentation should be avoided	NA	Yes	Yes
Posterior pollutant production should be avoided	Yes	No	No
Substrate addition needed	NA	Some cases	No
Oxygenation	Some cases	Some cases	No

Table 4. Advantages and features of some remediation strategies.
NK=Not known, NA=not apply

Due to the exchange of gases, water and metabolites between plants and their surroundings, plants could be used for soil, water or air bioremediation. Water bioremediation could be achieved off-site by filtration throughout soil with bioremediators or *in situ* by aquatic plants able to bioaccumulate metals. Having the plant-rhizosphere ecology, phytoremediation encompasses the microorganism and the plant biodegradation. Moreover, plants exert biological control on rhizosphere biodiversity and quantity; in the same way, fungi and bacteria control them as a result of allelopathy and all the competitive interactions

between rhizosphere microorganisms. In understanding of this, it is reasonable that phytoremediation takes more time than microbioremediation, but the former requires no substrate input and generates fewer sub-products. This suggests that phytoremediation could be a more environmental friendly technology than microbioremediation.

Few bioremediators have been found for each pesticide. Certainly, a bioremediators would not biodegrade all kind of pesticides, or even the same kind of pesticides to which they were proved to bioremediate. To illustrate, it cannot be assumed that a parathion bioremediator will also efficiently biodegrade other organophosphates, let alone other kind of pesticides like organochlorine. Therefore, for each pesticide, adequate bioremediators have to be found. Furthermore, to avoid bioagumentation, it is essential to find the most satisfactory bioremediators.

7. Conclusions

The choice of the bioremediation strategy should be made on the basis of type of pesticide, environmental matrix and the organisms present in the ecosystem. Since, the organism is the only eligible factor, the knowledge about features, advantages or disadvantages of organisms could be a decisive factor on bioremediation proficiency. Some parameters have to be addressed to assure bioremediation. In bacteria and fungi, pH, temperature, cell count, biomass growth rate, substrate bioavailability, and moisture are some of them. Plants require less supervision, but finding the best phytoremediator could be a hard and time-consuming task. Derivative bioremediation is a promising strategy. To get all the benefits from this strategy is necessary to carefully select the most adequate enzyme, and to have it well-characterized. Regardless, further research on biodegradation or biotransformation mechanisms in plants, bacteria, fungi or algae is imperative if bioremediation strategies are to be implemented or improved.

Author details

Jesús Bernardino Velázquez-Fernández*, Maricela Ramírez-Sandoval
and Delia Domínguez-Ojeda
Laboratory of Toxicological Biochemistry, Autonomous University of Nayarit, Mexico

Abril Bernardette Martínez-Rizo
Laboratory of Physiology, Autonomous University of Nayarit, Mexico

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* Corresponding Author

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