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Nature-based remediation of pesticides (running title)

Bio- and phytoremediation of pesticide contaminated environments: a review

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Abstract

Pesticide-contaminated fields can be found worldwide due to excessive use of insecticides, herbicides and fungicides. Many of the pesticides that were once used intensively are now forbidden and were shown to cause deleterious health effects.

Plants, bacteria and fungi have been revealed to possess pesticide-degrading capacities which can be applied in the successful remediation of contaminated fields and water.

This review will first provide an overview of the different types of pesticides, their application, and their key characteristics, followed by an analysis of their behaviour in the environment. Pesticides that are introduced into the environment seldom stay where they were applied. A complex system of transport, transfer, and transformation of pesticides throughout different environmental compartments often takes place. These processes all influence the possible remediation of the pesticide-contaminated media. Then several possible remediation strategies that are currently available will be discussed. Bioremediation is the first technology that is reviewed. With bioremediation, the focus is on the remediation of pesticides by microorganisms in bulk soil, without the aid or presence of plants. Secondly, plant-associated remediation is discussed. When focusing on plant-associated remediation, a distinction has to be made between rhizoremediation in the rhizosphere and phytoremediation within the plant tissues. While rhizoremediation and phytoremediation processes are possible solely with the use of plants, many of these processes are optimized by associations between plants and microorganisms. Plants and bacteria or fungi often live in a symbiotic relationship that aids them in surviving contaminated environments, as well as with the degradation of the contaminants they encounter.

In the last part of the review, we discuss the advantages and disadvantages of “natural” remediation strategies as compared to more classical industrial approaches.

1. Introduction

Pesticides have been extensively used worldwide for crop protection in agriculture and gardening as well as in the management of insect-borne diseases such as malaria and typhus [1]. This widespread use has led to the contamination of many agricultural soils, natural water reservoirs and rural areas by persistent organic pesticides [2, 3]. For a long period, the primary goal in farming was to protect crops against pests and thus gain the highest crop yields possible. In the meantime, the toxicity of the compounds used to both the farmers and consumers of the crops, as well as the environment, were likely underestimated and not always the primary concern when applying pesticides [4]. Many of the pollutants were applied globally for years before it was discovered that they possessed unacceptable toxicity and hazard with regard to human health [5]. Often, these chemicals were also persistent in natural environments. Long after their original use, the analytes remain in soils and sediments, from which they can subsequently enter the food chains and surface and ground water [6, 7]. One primary concern for these persistent organic pollutants (POPs) is that their hydrophobicity can lead to accumulation in adipose tissues of animals, which can cause biomagnification in higher trophic levels. These increasing levels of toxic compounds in the body may cause health problems over time [8, 9].

Classical remediation technologies for areas contaminated with POPs include physicochemical methods such as incineration, burning, land filling, composting and chemical amendments [10, 11]. As these methods are mainly *ex situ*, there is a high cost associated with excavation and transportation. Furthermore, since a significant part of the soil is removed, these methods are invasive and destructive to the overall ecosystem. Consequently, over the last decades there has been increasing interest for *in situ* remediation technologies, since they are less invasive, low-cost, low-maintenance and often solar-driven [3]. Bioremediation and phytoremediation, either with or without the assistance of plant-associated bacteria, are two “natural” remediation technologies that have been proven successful in many instances. Both strategies rely on the natural capacities of soil microorganisms and plants to take up contaminants as they do with nutrients and to metabolize, store or even co-metabolize them [12]. The efficient application of these technologies is complicated

by the wide variety of contaminants that are present in soils. Since every contaminated soil has its own specific physicochemical properties and contaminant profile, each remediation process has to be optimized or even tailored accordingly [13]. Depending on the situation, a choice has to be made whether to utilize bioremediation, which relies solely on soil microorganisms such as bacteria and fungi, or for phytoremediation, which relies on plants for the remediation of contaminated soils. A third option is to exploit the symbiotic relationships between plants and their associated microorganisms for an enhanced phytoremediation efficiency of POPs and other contaminants [14]. In this review, an overview will be given on the common remedial options for different pesticides. Bioremediation and phytoremediation, with or without plant-associated microorganisms, are both discussed in detail and a summary of plant and microbial species that have proven effective in remediation is provided. Lastly, the future challenges and perspectives are described.

2. Pesticides: Different types and characteristics

“Pesticides” is the collective term for all chemicals that are used to counteract a certain group of organisms. The primary classes of pesticides are insecticides (against insects), herbicides (against plants) and fungicides (against fungi). However inside these different classes, there still is a wide variety between the compounds and their chemical properties. They are defined by their ionic or non-ionic properties, hydrophobic properties, mechanism of action, and their molecular structure [15].

The 4 main groups of insecticides are: organochlorines, organophosphates, carbamate esters and pyrethroids. When considering insecticide contamination, organochlorines are often the chemicals of greatest concern. Commonly known members of this group are the DDTs (DDT = dichlorodiphenyltrichloroethane, DDE = dichlorodipenyldichloroethylene, DDD = dichlorodipenyldichloroethane), the HCHs (hexacyclochlorohexanes: α , β , γ , δ , t) and chlordane [2]. No less than eight compounds of the “Dirty Dozen” that were defined by the Stockholm Convention on POPs are organochlorine insecticides (Annex A, Stockholm Convention, 2001). The Stockholm

Convention states that these compounds are banned and that remediation for their presence in the environment is needed. Many soils are contaminated with low to moderate levels of DDT and its breakdown products, although the use of this compound has been forbidden for decades [16]. These molecules are highly hydrophobic, with log K_{ow} values between 5.5 and 6.9. When these compounds reside in soils for decades, a significant weathering effect can be observed [17]. Weathered DDTs strongly adsorb to soil particles, further enhanced by alternate drying and wetting. Due to their hydrophobic and lipophilic nature, DDTs naturally accumulate in adipose tissue and are often get magnified in the food chain. Several higher trophic level animals have been shown to experience deleterious effects of DDT exposure, e.g. egg shell thinning in birds and endocrine disruptors in mammals [18].

The most widespread herbicide contamination occurs with atrazine [2]. Atrazine (2-chloro-4-(aminoethyl)-6-(aminoisopropyl)-s-1,3,5-triazine) is a photosynthesis-inhibiting herbicide and is used in agriculture for the control of annual grasses and broad-leaved weeds, as well as in industrial sites and along railroads [19]. This caused widespread contamination of surface and groundwater reservoirs. Atrazine is known to be an endocrine disruptor, and significant toxicity has been reported in amphibians [20, 21].

Less is known about fungicide contamination, but the most important contaminants are hexachlorobenzene (HCB) and pentachlorophenol. Hexachlorobenzene is a hydrophobic organic compound that is known for its bioaccumulation and the analyte has been detected in air, soil, fish, birds, and even human milk [22]. Although HCB has not been produced since the 1970s, it is still being released into the environment as a by-product of simazine and thus, overall contamination has been increasing [23].

3. Behaviour of pesticides in the environment

The behaviour of pesticides in the environment is influenced by a combination of natural processes. In the environment pesticides interact with soils, water and organisms and the interactions are

controlled by a complex collection of biological, physical and chemical reactions [15]. Generally speaking, the processes that influence pesticide contamination can be classified into three types: (1) transport processes, which move the pesticide from the original point of introduction, (2) transfer processes, which control the pesticides movement through environmental compartments such as water, sediments, the atmosphere and biota and (3) transformation processes that change the structure/nature of a pesticide or even completely degrade it to its constituent elements. All these processes are in turn influenced by different soil and climatic factors, as well as the characteristics of the pesticides themselves. The most important soil factors are the soil structure, soil depth, pH and organic matter content as well as the soil slope. Soils that have a fine texture have a larger surface to volume ratio and a lower permeability. Therefore, the water and pesticides tend to diffuse more slowly, giving the pesticide a longer time for sorption to soil particles. Soils containing larger particles will retain considerably less pesticide residues. Soil depth influences the period of time the pesticides spend in contact with soil particles before being washed out to deeper layers. Soil pH has an effect on the adsorption potential of soils and the rate of the biological processes that can remove pesticide residues. Soil organic matter content can change the sorption potential towards pesticide molecules, as well as the number of biologically active microorganisms [24]. Organic matter provides an energy source for the microorganisms during the possible degradation of pesticide residues and is a highly complex medium that can interact significantly with organic and inorganic analytes. Finally, soil slope is an external geological factor that can change the time that water and pesticides have to infiltrate the soil. Steep areas have considerably more runoff, giving pesticides less time to seep through.

The climate can also have an effect on the remediation of pesticides in the environment. A well-studied example is weathering of pesticides. Weathering is the process where pesticides molecules become more firmly attached to soil particles through the process of becoming wet by rain or dew and drying again [25]. Weathered pesticides are known to be accumulated to a lesser extent than their recently applied equivalent.

In addition to soil and climatic factors, the chemical characteristics of the pesticide itself will greatly influence the fate of these compounds in the environment. Important characteristics include water solubility, tendency to adsorb to the soil particles and the half-life period or persistency in the environment.

The contaminant's availability is determined by combining the above-mentioned soil and pesticide characteristics. Contaminant availability refers to the rate and the extent that the pesticide molecules will be released to and remain in the environment and greatly influences the possible remediation potential of a certain technology.

3.1. Transport of pesticides

Transport of pesticides is the movement of the compounds from their point of introduction to the environment [15]. Most pesticides are applied by spraying, causing a partial evaporation of the analytes into the air. Secondly, pesticides can evaporate from the soil and from plant surfaces. When the pesticides interact with soil, the fraction of the analyte that does not adsorb to soil particles can leach through the matrix into surface waters and cause contamination there.

3.2. Transfer of pesticides

In addition to transport through the environment, pesticides can also be transferred between the different individual compartments: soil, water, atmosphere and biota [15].

Originally, most pesticides are applied in solid or liquid state to soils and plant surfaces, but volatilization can take place. Volatilization is a process where solid and liquid pesticides are converted into the gas phase, with subsequent transfer to the air [26, 27]. Once in the air, the pesticides can be transported over long distances and contamination can be spread throughout larger areas.

Precipitation events will partially convert the solid and liquid pesticides into dissolved forms. Part of this water will run off the soil, which can cause pesticide contamination in the bodies of water where

the runoff accumulates. However, a significant part of the rainwater will also enter the soil system at a distance from the site of application; in this case, leaching occurs. The amount of leaching that takes place is highly dependent on both the pesticide properties and the geological conditions.

From soil, air or water, pesticides can also be transferred into plants, microorganisms and animals. This capacity of plants and microorganisms to accumulate pesticides is the foundation of the natural remediation technologies that will be discussed in detail below.

3.3. Transformation

A third process to which pesticides are susceptible in the environment is transformation [15]. Pesticide transformation or degradation is the oxidation of pesticide molecules. When a pesticide is introduced into the environment, it is prone to different transformation pathways. First, there is a chemical degradation, where the analytes react with organisms or enzymes in the environment and degradation occurs. Secondly, the molecules can be degraded by exposure to light, *i.e.* photodegradation. Thirdly, microbial degradation can occur in both bulk and rhizosphere soil. The microbial degradation process is the basic process for bioremediation of pesticide-contaminated soils and will be further explored below.

4. Remediation of pesticides

Remediation strategies for soils and waters contaminated with pesticides can be physical, chemical, biological or a combination of some or all of these approaches [2]. Traditionally, pesticide contamination has been remediated using physico-chemical technologies where soils are excavated and subsequently transported to specialized landfills; soils may also be incinerated or stabilized on site [28]. Although efficient, these technologies have significant limitations. Excavation and transport of contaminated soils are both labour-intensive and costly. Furthermore, when soils are treated in this manner, ecosystem disruption is significant and recovery may take years [29]. Another limitation to these technologies is the area on which they can be applied. Since they are so costly, intensive,

and invasive, they are only cost-effective for small areas of high contamination; use on large areas with moderate contamination is not feasible [30].

Viable alternatives to these traditional remediation technologies are bio- and phytoremediation. These approaches are innovative technologies that show promise for alleviating pesticide contamination in both soils and water [31]. An overview of the main concepts underlying bio- and phytoremediation follows below.

4.1. Bioremediation

Bioremediation is the partial or complete conversion of the contaminant of interest to its elemental constituents by soil microorganisms [32]. It is estimated that one gram of bulk soil contains more than 1 million bacterial cells of 5000-7000 different species and more than 10,000 fungal colonies [33, 34]. The metabolic potential of the indigenous microbial community can be used for the detoxification of pesticide residues in soil [35-37].

The efficiency of bioremediation depends on the bioavailability of the contaminant (related to analyte adsorption to solid materials and to surface complexation) and on the degradation potential of the microorganisms [3]. Here, we review the relevant bioremediation and in these articles, many microorganisms showing pesticide-degrading capacities are described as shown in Table 1 (bacteria) and Table 2 (fungi).

When considering the bacteria, a dominant presence of the Proteobacteria is clearly evident. Of the 35 bacterial species that were recently reported to have the ability to remediate pesticide contamination, 21 belong to the Proteobacteria (6 Alphaproteobacteria, 4 Betaproteobacteria, and 11 Gammaproteobacteria). When focusing on the genus level, *Pseudomonas* sp. is the most abundant group present in literature. Species of this genus were mentioned in 16 publications and were shown to facilitate the remediation of 25 different pesticide residues and metabolites. The *Pseudomonas* species that were described were isolated from bulk soils, as well as the rhizosphere and from plants themselves, indicating that this species is both omnipresent and adaptable.

Significantly less has been published on the possible remediation of pesticides using fungal species (Table 2). A total of 13 fungal species have been reported, of which 7 belonged to the Ascomycota, 5 to the Basidiomycota and 1 to the Glomeromycota (Coninx et al., this issue). Many of these species were isolated from decaying wood (white rot fungi) and bulk soils. The large difference in the number of studies focusing on bacteria and might be the result of: (1) fungi showing less pesticide-degrading potential or (2) fungi are simply less studied in the context of natural remediation technologies.

We will review the most common pesticides. Chlorpyrifos is an organophosphorus pesticide that has been used in agriculture and can be very persistent in soils. However, many bacterial and fungal strains have shown potential for degrading this compound [38]. *Serratia* sp. was shown to completely degrade 100 mg L⁻¹ of chlorpyrifos in as little as 18 hours [39]; *Stenotrophomonas* sp. demonstrated similar results in 28 hours [40]. Gilani *et al.* [38] identified 14 different *Pseudomonas* strains isolated from soil that degraded chlorpyrifos. Many soils, such as those from former pesticide production facilities, are contaminated with mixtures of pollutants [41, 42]. To remediate these contamination scenarios, researchers may have to use consortia of different bacterial strains such as that employed by Fan *et al.* [43] for the degradation of atrazine and deisopropylatrazine. Alternatively, one may apply bacterial strains that can degrade several pesticides, such as the *Bacillus* strain used by Myresiotis *et al.* [44] to remediate soil contaminated with acidobenzolar-S-methyl, metribuzin, napropamide, propamocarb hydrochloride and thiamethoxam.

The efficiency of bioremediation processes depends largely on the local environmental conditions, such as soil moisture, redox status, temperature, pH and organic matter content [45]. The soil moisture is determined by the soil water content and not only influences the availability of water to soil microorganisms, but also on the redox conditions that can impact possible biochemical degradation reactions [46]. Furthermore, high soil water content can create anoxic conditions, which can alter the microbial activity [47]. For example, anoxic conditions can enhance the degradation of pesticides, as demonstrated by Phillips *et al.* [47] for HCH and by Wu *et al.* for DDT [48].

Temperature and pH are also two major parameters that influence biodegradation processes in soils [49]. Like most other enzymes, those molecules demonstrated to be involved in the degradation of pesticides are known to be temperature dependent [50]. Temperatures ranging between 15°C and 40°C were shown to be optimal conditions for the degradation of pesticides such as fenitrothion [51] and fenamiphos [45]. Enzymatic activity is also dependent on pH. Most bacteria function optimally in a pH range between 6.5 and 7.5, conditions that approximate the intracellular pH [31]. In addition to affecting enzyme activity, soil pH can also influence abiotic adsorption and desorption processes of pesticides in soils. Lowering soil pH can increase pesticide desorption from soil particles [52], which enhances the bioavailability and bioremediation efficiency.

Another factor influencing the adsorption and desorption of pesticide molecules to soil particles is the soil organic matter content. When soils contain higher levels of organic matter, two competing effects are possible. On the one hand, pesticides may bind more strongly to organic soil particles and thus become less accessible for biodegradation. Alternatively, in high organic matter soils more nutrients may be available for the soil microorganisms, which can then stimulate microbial growth and an increase in pesticide degradation [53]. Zhang *et al.* [54] made a comparison between the inhibition by adsorption and the stimulation by nutrient presence in benzonitrile-contaminated soils and concluded that the contaminant degradation increased with the addition of wheat-derived char. However, the more hydrophobic compounds with a higher log K_{ow} were likely to become more strongly associated with the solid phase of the organic matter [55]. Many soil microorganisms produce organic acids, which may cause the desorption of pesticide molecules from organic matter particles and thus increase biodegradation potential [56, 57].

Since microorganisms are capable of excreting large amounts of surfactants and enzymes, it is possible that the pesticides are also degraded extracellularly by these released enzymes [31]. This strategy could be exploited and serve as a rapid method for the remediation of pesticide-contaminated soils since no energy-demanding processes to transport the analytes into biota are needed. In fact, some researchers have reported successful bioremediation experiments using free

enzymes [58-61]. However, their application under field circumstances and optimization for site-specific environmental factors has yet to be deployed on a large scale.

4.2. Plant-associated remediation

Phytoremediation is based on the same principles as bioremediation, although in this case pesticide degradation takes place in the plant or its rhizosphere. Similar to bioremediation, phytoremediation is also to be considered to be an innovative, cost-effective and ecologically beneficial technology [31]. Phytoremediation is a collection of processes, including phytotransformation, phytodegradation, phytovolatilization and rhizoremediation (Figure 1). For the first three processes, uptake of the contaminant into the plant tissues is necessary; rhizoremediation takes place at the soil-root interface or rhizosphere.

All of the above-mentioned phytoremediation processes can be influenced by plant-associated microorganisms. Both rhizospheric and endophytic microorganisms can play a role in the remediation through pesticide-degrading and plant growth-promoting capacities [13, 62, 63].

[INSERT FIGURE 1 HERE PLEASE]

4.2.1. Rhizoremediation

The degradation of pesticides is often greater in rhizosphere soil than in bulk soil. This can be explained by a phenomenon known as the rhizosphere effect [31]. The rhizosphere is the soil volume directly around the roots and is heavily influenced by the activities of the plant. This activity makes the rhizosphere a more complex environment than bulk soil, supporting large numbers of metabolically active microbial communities. These numbers can be 10-100 times larger than the number of microorganisms in unvegetated or bulk soil [2, 14, 64], reaching up to 10^{12} cells per gram of soil [65]. Notably, the presence of plants with a large rhizospheric community can even increase the number of microbial cells in surrounding bulk soils [66, 67]. The presence of microbial

communities in the soil can be beneficial to the plant by producing protective or beneficial compounds such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase [13]. ACC is the immediate precursor molecule for ethylene, which is a stress hormone that plants often produce when growing on contaminated soils. When ACC-deaminase cleaves ACC, ethylene levels are lowered and the plants experience less stress from contaminant exposure. All of these mechanisms can decrease plant phytotoxicity, increase plant growth and increase phytoremediation potential. Furthermore, microorganisms are capable of facilitating the uptake of essential nutrients by plants through the production of organic acids [68]. The microbiota may also protect plants against pathogens by competing for a position in the plant microbiome and degrade contaminants before they negatively affect the plant [69].

Rhizodegradation is a process that occurs naturally, but that can be enhanced by planting the most appropriate plant species, or by adding pesticide-degrading bacteria through inoculation. Plant root systems can excrete enzymes that degrade pesticides in the rhizosphere [69], but they also release photosynthetic products that can serve as carbon and energy source for rhizospheric bacteria. Several researchers have isolated rhizospheric bacteria that show pesticide-degrading capacities (Table 1). If these pesticide-degrading bacteria are enriched by means of inoculation, the process is considered to be bacteria-enhanced rhizodegradation. Kidd *et al.* [70] showed a higher dissipation of HCHs in the rhizosphere when *Cytisus striatus* and *Holcus lanatus* were inoculated with HCH-degrading bacteria. Ahmad *et al.* [71] reported a 50% increase in chlorpyrifos degradation in the *Lolium multiflorum* rhizosphere when the plant roots were inoculated with *Bacillus pumilus* C2A1.

Wang *et al.* [72] described the successful degradation of phoxim when carrot (*Daucus carota*) and green onion (*Allium fistulosum*) were inoculated with the arbuscular mycorrhizal fungi *Glomus intraradices* and *Glomus mosseae*; notably, contaminant degradation was negligible in non-inoculated plants. The fungus *Trichoderma harzianum* was isolated from a marine plant *Didemnum ligulum* and had the capacity to degrade 50 mg L⁻¹ phoxim in liquid medium in 7 days [73]. Jauregui *et al.* (2003) conducted a large experiment on pesticide degradation by fungi. These researchers tested

17 white rot fungi; 16 of them showed able to degrade the pesticides parathion, terbufos, azinphos-methyl, phosmet, and tribufos after a four-day growth period.

Similar to bioremediation, rhizoremediation processes are also heavily influenced by plant, soil and pesticide characteristics. Factors such as temperature, pH and soil organic matter content influence pesticide bioavailability, as well as the bacterial and enzymatic degradation potential in the rhizosphere. However, the presence of the root system can moderate some of these effects and in general, the degradation potential is higher in comparison to bulk soil [31].

The rhizosphere can also play another role in phytoremediation processes: rhizostabilization. As explained earlier, pesticides can move through the soil as runoff from agricultural fields and subsequently contaminate surface waters. However, root systems and their associated microbial communities may intercept the pesticide molecules and thus stabilize them in the soil [2].

4.2.2. Phytoremediation

In contrast to rhizoremediation, accumulation of the pesticide is a prerequisite for phytotransformation, phytodegradation, and phytovolatilization. Many plants have been reported to efficiently accumulate pesticides; an overview is given in Table 3. *Cucurbita pepo* and *Zea mays* are the plant species most frequently used in research papers addressing phytoremediation of pesticides (11 and 7, respectively). These plants are often considered for phytoremediation because of their high number of cultivars as a result of their important role in agriculture and gardening, as well as their good accumulation potential of a wide range of organic contaminants [25, 74]. *Ricinus communis* is the plant species shown to accumulate the greatest number of different contaminants. Rissato *et al.* (2015) and Huang *et al.* (2011) described the uptake of 11 different pesticides with a wide variety of characteristics.

The plant's uptake efficiency of these compounds is determined by many soil and plant characteristics [31]. The soil factors that can influence the pesticide availability to microorganisms and plants were discussed earlier in this review. In addition to soil moisture, temperature, pH and

organic matter content, the time that the pesticide resides in soils can also influence the pesticide uptake; time-dependent decreases in availability are often described during weathering or aging of the residues [17, 68].

The potential of plants to take up pesticide residues varies greatly between plant species [17, 25, 41, 42, 75-82] and even between different subspecies [74, 83]. To maximize the phytoremediation potential in a contaminated field, the optimum combination of soil, plant and possibly endophytes has to be established. Bouldin *et al.* [84] tested two different plants (*Juncus effuses* and *Ludwigia peploides*) for their uptake potential of two different pesticides (atrazine and lambda-cyhalothrin) and observed a higher uptake of atrazine in *J. effuses*, while *L. peploides* accumulated more lambda-cyhalothrin. Atrazine was efficiently translocated to the shoots of *J. effuses*, but *L. peploides* showed a 98.2% retention of the analyte in the roots. The differences in uptake and translocation efficiency of certain plants towards pesticides depend on pesticide and plant characteristics. The log K_{ow} or octanol-water partitioning coefficient greatly influences the availability and translocation of pesticides in plants [85]. Turgut [86] investigated the uptake of trifluralin, atrazine, terbutryn and cycloxdim in *Myriophyllum aquaticum* and observed an increasing root concentration factor and submerged shoot concentration factor with an increasing log K_{ow} . The more polar (hydrophilic) a compound is, the more difficulties the analyte has in crossing biological membranes, which causes a lower uptake in comparison to lipophilic compounds that easily cross biomembranes [87-89].

In environments that are contaminated with several pollutants, significant interactions between the contaminants may occur. Su *et al.* [90] documented that the interaction between Cd^{2+} and atrazine reduced the individual toxicities of the contaminants to *Oryza sativa* seedlings and increased the uptake and translocation of atrazine into the plant tissues.

Plants mainly accumulate pesticides through a soil-to-plant pathway; although deposition from the air is also possible in the form of straight deposition from the gas phase or contaminants that are sorbed to particles that are subsequently deposited. Lee *et al.* [91] studied the uptake of chlordane

and reported different profiles of chlordane in *Cucurbita pepo* when taken up through air or soil in parallel studies.

In case of the soil-to-plant uptake, several plant characteristics such as water uptake potential and root depth/structure can influence the accumulation potential. Once pesticides are retained by plant root tissues, they can be immobilized in the roots or translocated to the aerial plant parts where the analytes can be stored, metabolized, or volatilized (Figure 1). Generally, pesticide accumulation in roots is inefficient for remediation purposes; although the soil contaminant concentration decreases, root tissues generally are not cost-effectively harvested. Aquatic plant-based remediation systems are an obvious exception to this, where contaminant removal by plant roots can be significant. *Eichhornia crassipes* (water hyacinth) can accumulate the insecticide ethion more efficiently in its roots than in its shoots. Given that the roots make up over 50% of the plant mass and that the total plant, including roots, can easily be harvested, this system can be efficiently used for the phytoremediation of ethion-contaminated waters [92].

After being accumulated by plant roots, pesticide molecules can be transported to the xylem vessels and translocated with the transpiration stream of the plants. Many studies have been devoted to the transport of pesticides within crop species, largely because of the plants' high growth rates and easy cultivability [93]. Several crops have been reported to show significant accumulation potential for a range of pesticides (Table 3). White *et al.* [25, 74, 94, 95] studied the uptake of DDE by different *Cucurbita pepo* cultivars and reported shoot bioconcentration factors up to 23.7 for the Raven cultivar. A fraction of the pesticide molecules that are translocated to shoots can be adsorbed within vessel macromolecules, such as lignin or cellulose. The use of trees, mainly poplar and willow, takes this mechanism into account for phytoremediation as well as for phytopumping [87, 96, 97]. Phytoremediation using solely phytoaccumulation requires harvest of the shoots after the uptake period. The shoot tissues can subsequently be burned or composted or otherwise disposed of by other means [2].

Pesticides that are volatile such as trifluthrin can be transported to the shoots and subsequently be volatilized to the atmosphere (Figure 1). This process is often an unwanted side effect of the transport processes in the plant, since the end result is merely a relocation of the contamination from soil to air; the exception would be if the plant evaporation were captured [2].

Generally, the aim of efficient phytoremediation is not solely phytoaccumulation, but also the metabolic breakdown of the contaminant within the plant tissues; this can be made with or without the aid of endophytic bacteria. Plants often metabolize pesticides into more polar molecular structures that can be stored in vacuoles or bound as residues in cell walls [98]. In rape (*Brassica rapa*), atrazine residues were shown to be incorporated into plant cell walls as hydroxyatrazine (HO-A) [99]. In roots, this process can lead to stabilization of the pesticides and therefore limit re-dispersion of the contaminants after plant death; in this scenario, the degrading capacity of white-rot fungi for lignin becomes very important [100, 101].

Few compounds can be completely degraded by only plant metabolic pathways since most pesticides contain one or more aromatic cycles that are inherently difficult to break. In this case, bacteria- or fungi-enhanced phytoremediation may become important. As discussed earlier, many bacteria and fungi show pesticide degrading capacities in soils; the same is true for endophytic microorganisms (Table 1 and 2). Xu *et al.* [102] showed that *Spirodela polyrhiza* stimulated the growth of their endophytes, which in their turn led to the degradation of fenpropathrin inside the plant tissues. Chen *et al.* [103] also concluded that the efficient remediation of waters polluted by a mixture of chlorpyrifos, fenpropathrin, naphthalene and bifenthrin was mainly due to the endophytic bacteria residing in the aquatic plants, *Phragmites communis*, *Potamogeton crispus*, *Nymphaea tetragona* and *Najas marina*, as opposed to the plants themselves. Endophytic bacteria that show pesticide-degrading capacities have an advantage over their non-degrading competitors in contaminated environments and tend to dominate the community in those scenarios [32].

Using endophytes in the phytoremediation process not only has an advantage when it comes to pesticide degradation; these endophytes also often possess plant growth-promoting properties. This

plant growth promotion is shown through enhanced cycling of nutrients such as nitrogen and phosphate [104]. Endophytes have also been shown to possess phosphate solubilization abilities [105, 106], indole-3-acetic acid production [107] and iron-binding siderophores production [108], and ACC-deaminase production [13]; therefore, they are able to enhance plant growth. Furthermore, the bacteria can also indirectly protect the plants. Symbiotic plant endophytes can prevent or lessen the deleterious effects of certain pathogens, often by outcompeting these organisms from the microbial community [109].

5. Advantages and disadvantages of natural remediation technologies

Bio- and phytoremediation show many advantages, which make them preferred over the classical physico-chemical remediation strategies. These alternative remediation technologies are based on the natural abilities of plants and microorganisms to utilize a wide range of organic compounds and to metabolize these analytes into harmless products such as carbon dioxide and water. These technologies exploit the natural ability of an environment to restore itself. The greatest advantage is that these technologies remediate the soils *in situ* without major disruptions in the environment. No excavation and transportation are needed, which makes these technologies inherently less costly and labour-intensive, as well as more readily acceptable by the public.

Phytoremediation has some additional advantages over bioremediation. In the case of microbe-enhanced phytoremediation, the plants and microorganisms provide protection and nutrients for each other. Therefore, it is easier to stabilize the remediation system when compared to bulk soil, where microorganisms have to compete to a greater extent to become established in the community. In addition, plants that are grown during phytoremediation provide stabilization of the soil and could potentially be used for green energy purposes.

Although the advantages of these technologies are obvious, some disadvantages do also exist. First, not all contaminants are susceptible to biodegradation. The contaminants can also be toxic to plants (Hendrix et al., this issue) and microorganisms that may not possess the required degradation

pathways. Secondly, if the parent compound is only partially degraded, products might appear that are more toxic and persistent than the original contaminant. And even though bio- and phytoremediation require less resources in the field than the classical remediation strategies, greater efforts are required to address the site-specific requirements of each contamination, plant and/or microorganisms scenario. Also, if degradation does not occur in the plants, the contaminant might be released into the environment again through evapotranspiration or through decaying tissues associated with natural senescence.

Lastly, the time period that is required for efficient decontamination of the soil has to be taken in consideration. As is the case when considering the remediation strategy, the treatment is also heavily dependent on the specific circumstances. The traditional thinking here is that bio- and phytoremediation approaches are slower [69], although some studies have demonstrated the opposite. Compennolle *et al.* (2012) [110] investigated a case study where a BTEX plume was remediated using poplar trees. In this study, the cost-effectiveness of phytoremediation is compared to that of classical remediation strategies such as pump-and-treat. These researchers concluded that both phytoremediation and pump-and-treat reached the remediation goal within one year of treatment, with phytoremediation being of lower cost. Therefore, though depending on the situation, natural remediation technologies can be the most cost-effective approach.

6. Conclusions, perspectives and challenges

The contamination of soils with pesticides is of major concern to the environment and public health. Although bioremediation and phytoremediation are efficient technologies that have significant potential, civil-engineering based remediation strategies are still being applied more frequently. Soils and plants host a wide range of microbial communities with many metabolic pathways that can be applied for an efficient degradation of pesticide residues in soil and water. Recent progress in both plant biotechnology and microbiology, such as next generation sequencing to identify and utilize total microbial communities, make these technologies more and more efficient. The findings that are

discussed in this review show that bio- and phytoremediation have been successfully applied in several field trials and that these technologies should see greater use in the remediation of pesticide-contaminated field sites.

To expand the scale and efficiency of these technologies, greater focus is needed on unravelling the elucidating mechanisms of bio-, rhizo- and phytoremediation in the relevant biota. New technologies such as next generation sequencing might be useful in this regard; by investigating the composition of the total microbial communities that are present in soils, rhizospheres and plants, an efficient method to introduce new microorganisms to the community may be developed. However, for every specific contaminated site, a specific plan has to be composed to appropriately address the location-specific characteristics. Additional research is needed to expand the knowledge base on how to efficiently translate successful lab trials into robust field applications.

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Table 1: Overview of the bacterial genera proven to show pesticide degrading capacities and their source of isolation.

Bacterial Genus	Pesticide	Isolated from	Reference
<i>Aeromonas</i> sp.	Chlorpyrifos, Fenpropathrin	<i>Phragmites communis</i>	[103]
	Carbaryl	Bulk soil	[111]
<i>Agrobacterium</i> sp.	Atrazine, Metalochlor	Rhizosphere <i>Andropogon gerardii</i>	[112]
<i>Anabaena</i> sp.	Butachlor	Bulk soil	[113]
<i>Arthrobacter</i> sp.	Cyhalothrin, Cypermethrin, DDT, HCH	Rhizosphere <i>Nicotiana tabacum</i>	[114]
<i>Azospirillum</i> sp.	Ethion	Sludge	[115]
	Chlorpyrifos, Cyanophos	Bulk soil	[116]
<i>Bacillus</i> sp.	Acibenzolar-S-methyl, metribuzin, napropamide, propamocarb hydrochloride, thiamethoxam	Bulk soil	[44]
	Bifenthrin, Chlorpyrifos	<i>Phragmites communis</i>	[103]
	Carbaryl	Bulk soil	[111]
	Chlorpyrifos	Bulk soil	[117]
		<i>Lolium multiflorum</i>	[71]
	Cypermethrin	Bulk soil	[118]
	Methylparathion	Bulk soil	[119]
	Monocrotophos	Bulk soil	[120]
	Trifluralin	Bulk soil	[121]
<i>Bordetella</i> sp.	HCHs	Bulk soil	[122]
<i>Brevibacterium</i> sp.	Phorate	Bulk soil	[123]
<i>Chryseobacterium</i> sp.	DDT	Bulk soil	[124]
<i>Corynebacterium</i> sp.	Carbaryl	Bulk soil	[111]
<i>Cupriavidus</i> sp.	Azoxystrobin	Bulk soil	[125]
<i>Enterobacter</i> sp.	Bifenthrin	<i>Phragmites communis</i>	[103]
	Chlorpyrifos	Bulk soil	[45, 126]
	DDE	<i>Cucurbita pepo</i>	[127]
	Fenamiphos	Bulk soil	[45]
<i>Flavobacterium</i> sp.	Bifenthrin	<i>Phragmites communis</i>	[103]
<i>Herbaspirillum</i> sp.	Trifluralin	Bulk soil	[121]
<i>Klebsiella</i> sp.	Bifenthrin, Fenpropathrin, Naphtalene	<i>Nymphaea tetragona</i>	[103]
	Chlorpyrifos	Sludge	[128]
	Fenpropathrin	<i>Spirodela polyrhiza</i>	[102]
	Trifluralin	Bulk soil	[121]
<i>Lactobacillus</i> sp.	Chlorpyrifos	Rice straw	[129]
	Chlorpyrifos, Coumaphos, Diazonin, Methylparathion,	Kimchi (food dish)	[130]

	Parathion		
<i>Lactococcus</i> sp.	Fenpropathrin	<i>Nymphaea tetragona</i>	[103]
<i>Mesorhizobium</i> sp.	Chlorpyrifos	<i>Lolium multiflorum</i>	[131]
<i>Methylobacterium</i> sp.	DDE	<i>Cucurbita pepo</i>	[132]
<i>Microbacterium</i> sp.	Chlorpyrifos, Fenpropathrin	<i>Phragmites communis</i>	[103]
<i>Morganella</i> sp.	Carbaryl	Bulk soil	[111]
<i>Nocardia</i> sp.	Sulfentrazone	Bulk soil	[133]
<i>Novosphingobium</i> sp.	2,4-Dichlorophenoxyacetic acid	Bulk soil	[134]
<i>Paenibacillus</i> sp.	Chlorpyrifos	<i>Nymphaea tetragona</i>	[103]
	Chlorpyrifos, Cyanophos	Bulk soil	[116]
	Fenpropathrin	<i>Najas marina</i>	[103]
<i>Paracoccus</i> sp.	Alachlor, Acetochlor, Butachlor, Metolachlor, Propisochlor	Bulk soil	[135]
	Chlorpyrifos	Sludge	[136]
<i>Pseudomonas</i> sp.	Aldrin, Dieldrin, Heptachlor, Heptachlor Epoxide	Bulk soil	[137]
	Atrazine, Metolachlor	Rhizosphere <i>Sorghastrum nutans</i>	[112]
	Bifenthrin	<i>Phragmites communis</i>	[103]
	Carbaryl	Bulk soil	[111]
	Chlorpyrifos	Bulk soil	[38, 126]
	Chlorpyrifos, Cypermethrin, Fenitrothion, Fenpropathrin, Methyl Parathion, Permethrin	Bulk soil	[138]
	Cyhalothrin, Cypermethrin, Fenpropathrin, Fenvalerate	Bulk soil	[139]
	2,4-Dichlorophenoxyacetic acid	<i>Populus trichocarpa x deltoides</i>	[140]
	Dimethoate, Malathion	Bulk soil	[141]
	Endosulfan	Bulk soil	[142]
	Ethion	Sludge	[115]
	Fenpropathrin	<i>Spirodela polyrhiza</i>	[102]
	Glyphosate	Bulk soil	[143]
	Phorate	Bulk soil	[144]
	Quinalphos	Bulk soil	[145]
<i>Pseudoxanthomonas</i> sp.	Profenos	Bulk soil	[146]
<i>Psychrobacter</i> sp.	Chlorpyrifos	Bulk soil	[147]
<i>Rhodanobacter</i> sp.	Azoxystrobin	Bulk soil	[125]
<i>Rhodococcus</i> sp.	HCHs	<i>Cytisus striatus</i>	[148]
	Simazine	Bulk soil	[149]
<i>Serratia</i> sp.	Monocrotophos	Bulk soil	[150]
	Quinalphos	Bulk soil	[145]
<i>Sphingomonas</i> sp.	Acetachlor, Alachlor, Butachlor	Sludge	[151]
	DDE	<i>Cucurbita pepo</i>	[152]

	Diclofop-methyl	Bulk soil	[153]
	HCHs	Bulk soil	[148]
<i>Stenotrophomonas</i> sp.	Chlorpyrifos, Diazonin, Methyl parathion, Parathion, Phoxim, Profenofos, Triazophos	Sludge	[154]
	DDT	Bulk soil	[155, 156]
<i>Streptomyces</i> sp.	Atrazine	<i>Saccharum officinarum</i>	[157]
	Chlordane, HCHs, Methoxychlor	Bulk soil	[158]
	HCHs	Bulk soil	[159]
<i>Variovorax</i> sp.	Linuron	Bulk soil	[160]

Table 2: Overview of the fungal genera known to degrade pesticides and their source of isolation.

Fungal genus	Pesticide	Isolated from	Reference
<i>Aspergillus</i> sp.	Pentachlorophenol	<i>Didemnun ligulum</i>	[73]
<i>Bjerkandera</i> sp.	Azinphos methyl, Phosmet, Terbufos, Tribufos	Wood	[100]
<i>Cladosporium</i> sp.	Pentachlorophenol	<i>Didemnun ligulum</i>	[73]
<i>Cordyceps</i> sp.	Dieldrin	Bulk soil	[161]
<i>Coriolus</i> sp.	Aldicarb, Alachlor, Atrazine	Bulk soil	[162]
<i>Fusarium</i> sp.	Pentachlorophenol	<i>Didemnun ligulum</i>	[73]
<i>Glomus</i> sp.	Phoxim	<i>Medicago sativa</i>	[72]
<i>Lentinula</i> sp.	Difenoconazole, Pendimethalin, Terbutylazine	Bulk soil	[163]
<i>Penicillium</i> sp.	Sulfentrazone	Bulk soil	[133]
<i>Phanerochaete</i> sp.	Azinphos methyl, DDT, Phosmet, Terbufos, Tribufos	Wood	[100, 101]
<i>Pleurotus</i> sp.	Azinphos methyl, Phosmet, Terbufos, Tribufos	Wood	[100]
<i>Trichoderma</i> sp.	Pentachlorophenol	<i>Didemnun ligulum</i>	[73]
<i>Verticillium</i> sp.	Chlorpyrifos	<i>Brassica chinensis</i>	[164]

Table 3: Overview of the plant species that have shown pesticide phytoremediation potential

Plant Species	Pesticide
<i>Achillea millefolium</i>	DDT [165]
<i>Acorus calamus</i>	Atrazine [76]
<i>Allium fistulosum</i>	Phoxim [72]
<i>Amaranthus caudate</i>	Dimethoate, Malathion [141] Glyphosate [143]
<i>Andropogon gerardii</i>	Atrazine, Metolachlor, Pendimethalin [112, 166]
<i>Arachis hypogaea</i>	DDE [25]
<i>Avena sativa</i>	HCHs [167]
<i>Brassica campestris</i>	Endosulfan [81]
<i>Brassica juncea</i>	DDE [25]
<i>Brassica napus</i>	DDE [25]
<i>Cabomba aquatica</i>	Copper sulphate, Dimethomorph, Flazasulfuron [168]
<i>Cajanus cajan</i>	DDE [25]
<i>Canna x hybrida</i>	Simazine [169]
<i>Chenopodium spp.</i>	HCHs [167]
<i>Cirsium arvense</i>	Chlordane [95]
<i>Cucumis sativus</i>	Chlordane [78] DDE [78] Endosulfan sulfate [170]
<i>Cucurbita pepo</i>	Chlordane [95] DDD, DDE, DDT [17, 42, 68, 78, 94, 171-173] Dieldrin, Endrin [83, 174] HCHs [42, 173]
<i>Cytisus striatus</i>	HCHs [70, 148, 167]
<i>Daucus carota</i>	DDD, DDE, DDT [165, 173] HCHs [173] Phoxim [72]
<i>Digitaria sp.</i>	Atrazine, Metolachlor, Trifluralin [175]
<i>Eichhornia crassipes</i>	Aldrin, Chlorpyrifos, DDT, Dieldrin, Endosulfan, Malathion, Methylparathion [176], Ethion [92]
<i>Elodea canadensis</i>	DDT [177] Copper sulfate, Dimethomorph, Flazasulfuron [168]
<i>Erigeron canadensis</i>	DDD, DDE, DDT [165]
<i>Festuca arundinacea</i>	DDT [17]
<i>Glycine max</i>	Azoxystrobin [79] DDD, DDE, DDT [42, 77] HCHs [42]
<i>Halimione portulacoides</i>	DDD, DDE, DDT [178]
<i>Helianthus annus</i>	Azoxystrobin [79] DDD, DDE, DDT [77]

<i>Holcus lanatus</i>	HCHs [70]
<i>Hordeum vulgare</i>	DDD, DDE, DDT, HCHs [42] Dodemorph, Tridemorph [179]
<i>Iris Pseudacorus</i>	Atrazine [76] Chlorpyrifos [180]
<i>Jathropa curcas</i>	HCHs [181]
<i>Juncus effusus</i>	Chlorpyrifos [182] Gamma-Cyhalothrin [84] Atrazine [84, 182]
<i>Juncus maritimus</i>	DDD, DDE, DDT [178]
<i>Kanthium strumarium</i>	DDD, DDE, DDT [165]
<i>Kochia sp.</i>	Atrazine [175, 183] Metolachlor, Trifluralin [175]
<i>Lactuca sativa</i>	Chlordane [95] Dimethoate, Malathion [141]
<i>Leersia oryzoides</i>	Atrazine, Diazonin, Permethrin [75]
<i>Lemna minor</i>	Copper sulphate, Dimethomorph, Flazasulfuron [168] Atrazine, Clofibric acid, 2,4-Dichlorophenoxyacetic acid, Picloram [184] Glyphosate, Isoproturon [185]
<i>Lemna punctate</i>	Atrazine, Chlofibric acid, 2,4-Dichlorophenoxyacetic acid, Picloram [184]
<i>Lolium multiflorum</i>	Atrazine [186] Chlorpyrifos [71, 131] DDE [25] DDT [17] Terbuthylazine [187]
<i>Lolium perenne</i>	2,4-Dichlorophenoxyacetic acid [188] Pentachlorophenol [189]
<i>Ludwigia peploides</i>	Atrazine, Gamma-cyhalothrin [84]
<i>Lupinus albus</i>	Chlordane [95] DDE [25]
<i>Lupinus angustifolius</i>	Atrazine, Fenamiphos, Isoproturon, Simazine [190] Carbaryl, Linuron, Permethrin [190, 191]
<i>Lycopersicon esculentum</i>	Chlordane [95]
<i>Lythrum salicaria</i>	Atrazine [76]
<i>Medicago sativa</i>	DDD, DDE, DDT [17, 77] Napropamide [192]
<i>Myriophyllum aquaticum</i>	Atrazine, Cycloxidim, Tertbutryn, Trifularin [86] DDT [177] Simazine [169]
<i>Najas marina</i>	Bifenthrin, Chlorpyrifos, Fenpropathrin, Naphtalene [103]
<i>Nasturtium officinale</i>	Dimethoate, Malathion [141]
<i>Nicotiana tabacum</i>	HCHs [193] Sulfentrazone [52]
<i>Nymphaea tetragona</i>	Bifenthrin, Chlorpyrifos, Fenpropathrin, Naphtalene [103]

<i>Oenothera biennis</i>	DDD, DDE, DDT [165]
<i>Orychophragmus violaceus</i>	DDD, DDE, DDT, HCHs [194]
<i>Oryza sativa</i>	DDT [195]
<i>Panicum virgatum</i>	Atrazine, Metolachlor, Pendimethalin [112, 166]
<i>Phaseolus vulgaris</i>	DDD, DDE, DDT, HCHs [42] Dimethoate, Malathion [141]
<i>Phragmites australis</i>	DDT [195] Hexachlorbenzene [196]
<i>Phragmites communis</i>	Bifenthrin, Chlorpyrifos, Fenpropathrin, Naphtalene [103]
<i>Pisum sativum</i>	2,4-Dichlorophenoxyacetic acid [140]
<i>Plantago lagopus</i>	DDD, DDE, DDT [165]
<i>Plantago major</i>	Azoxystrobin [79] Chlorpyrifos [197] Cyanophos [116]
<i>Polygonum sp.</i>	Atrazine, Metolachlor, Trifluralin [175]
<i>Populus deltoides x nigra</i>	Atrazine [87]
<i>Potamogeton crispus</i>	Bifenthrin, Chlorpyrifos, Fenpropathrin, Naphtalene [103]
<i>Potentilla argentea</i>	DDD, DDE, DDT [165]
<i>Raphanus sativus</i>	DDT, HCHs [198]
<i>Ricinus communis</i>	Aldrin, Chlordane, Chlorpyrifos, DDE, Diclofop methyl, Dieldrin, Endrin, HCHs, Heptachlor, Methoxychlor [199] DDT [199, 200]
<i>Salix alba</i>	Metalaxyl, Trifluralin [96]
<i>Salix humboldtiana</i>	DDD, DDE, DDT [97]
<i>Sambucus nigra</i>	Metalaxyl, Trifluralin [96]
<i>Scenedesmus obliquus</i>	Isoproturon, Dimethomorph, Pyrimethanil [80]
<i>Scenedesmus quadricauda</i>	Isoproturon, Dimethomorph, Pyrimethanil [80]
<i>Schoenoplectus californicus</i>	Aldrin, Chlordane, DDD, DDE, DDT, Dieldrin, Endosulfan, HCHs, Heptachlor, Heptachlor epoxide [201]
<i>Scirpus maritimus</i>	DDD, DDE, DDT [178]
<i>Sedum alfredii</i>	DDD, DDE, DDT [155]
<i>Sesamum indicum</i>	HCHs [202]
<i>Solanum lycopersicum</i>	DDD, DDE, DDT [77]
<i>Solanum nigrum</i>	HCHs [167]
<i>Sorghastrum nutans</i>	Atrazine, Metolachlor, Pendimethalin [112, 166]
<i>Sorghum bicolor</i>	DDD, DDE, DDT, HCHs [173]
<i>Sparganium americanum</i>	Atrazine, Diazonin, Permethrin [75]
<i>Spinacia oleracea</i>	Chlordane [95] HCHs [203]
<i>Spirodela polyrhiza</i>	Fenpropathrin [102]
<i>Taraxacum officinalis</i>	DDD, DDE, DDT [165]
<i>Trifolium incarnatum</i>	DDE [25]
<i>Trifolium pretense</i>	2,4-Dichlorophenoxyacetic acid [188]
<i>Triticum vulgare</i>	Butachlor [204]

	DDD, DDE, DDT, HCHs [42]
<i>Typha latifolia</i>	Atrazine, Diazonin, Permethrin [75] Hexachlorobenzene[196] Methyl parathion [205]
<i>Vicia villosa</i>	DDE [25]
<i>Vigna radiate</i>	Aldicarb [82]
<i>Vigna sinensis</i>	Endosulfan sulfate [170]
<i>Vigna unguiculata</i>	Aldicarb [82]
<i>Withania somnifera</i>	HCHs [206]
<i>Zea mays</i>	Aldicarb [82] DDD, DDE, DDT [173] Endosulfan [81] Endosulfan sulfate [170] HCHs [159, 173] Napthalene [82]

Figure 1: Overview of processes included in bio- and phytoremediation.