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Biological Remediation of Phenoxy Herbicide-Contaminated Environments

Magdalena Urbaniak and Elżbieta Mierzejewska

Abstract

Phenoxy herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) are widely used in agriculture to control broadleaf weeds. Although their application has helped to increase the yield and value of crops, they are also recognized as a source of emerging environmental contamination. Their extensive use may promote contamination of soil, surface, and groundwater and lead to increased inhibition of plant development and soil toxicity. Hence, there is an urgent need to identify nature-based methods based on appropriate biological remediation techniques, such as bio-, phyto-, and rhizoremediation, that enable the effective elimination of phenoxy herbicides from the environment. Bioremediation typically harnesses microorganisms and their ability to utilize recalcitrant contaminants in complete degradation processes, while phytoremediation is a cost-effective, environmentally friendly strategy that uses plants to transform or mineralize xenobiotics to less or nontoxic compounds. Rhizoremediation (microbe-assisted phytoremediation), in turn, is based on the interactions between plant roots, root exudates enriched in plant secondary metabolites, soil, and microorganisms. Based on the above, this chapter presents current knowledge on the properties of phenoxy herbicides, as well as the concentrations detected in the environment, their toxicity, and the biological remediation techniques used for safe removal of the compounds of interest from the environment.

Keywords: 2,4-D, MCPA, bioremediation, phytoremediation, rhizoremediation, toxicity, degradative genes

1. Phenoxy herbicides: general information

2,4-Dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) are the most commonly used phenoxy acid herbicides in agriculture, and 2,4-D is now the fifth most extensively used active ingredient (a.i.) in the US agricultural and home/garden market sector [1]. In addition, in 2016, 6.5 mln kg of herbicides based on phenoxy-phytohormones (2,4-D and MCPA) were sold in in the EU, including ~2 mln kg sold in Poland [2].

Phenoxy herbicides are typically used to protect wheat, one of the most extensively cultivated crops, because they selectively control the growth of dicotyledonous weeds [3]. They are applied as post emergence agents and taken up by broad-leaved plants. 2,4-D has also been extensively used as an anti-stalling agent for the postharvest fresh fruit industry [4].

These herbicides are based on ring-like structures and have at least one chlorine atom attached to the ring at different positions [5]. Their action is similar to that of phytohormones (auxins) insofar that they can redirect the regulation of plant growth/physiological processes, resulting in nutrition deficiency and subsequent plant death [6].

They are typically released to the environment in the form of commercial products containing phenoxy acids salts or esters; however, they immediately hydrolyze to their corresponding anionic or neutral form [7]. The dosage of phenoxy herbicides lies in the range of 0.8–1.8 kg of a.i. per ha. Their transport through the environment is governed by soil and climate factors (e.g., distribution of soil particles, soil permeability, soil depth, soil pH, soil organic matter content, land slope) [8], and their retention and translocation in the soil profile also depend on their chemical and physical properties, which are described by several parameters (**Table 1**), particularly pK_a (acid dissociation constant), logP (octanol-water partition coefficient), and K_{oc} (organic carbon distribution coefficient). The degree of adsorption and desorption depends on time and the physicochemical properties of soil; however, 2,4-D and MCPA are rather poorly adsorbed on the soil particles in comparison to their derivatives, which have different sorption characteristics [7].

Although phenoxy herbicides are described as nonpersistent and weakly adsorbed ($K_{oc} < 50$) in soil, they can be transported with runoff and in soil profile and reach terrestrial and water ecosystems (surface and groundwater). **Figure 1** summarizes the transport and transfer processes of phenoxy herbicides in the environment. After they are applied to land, they are spread through several processes, including sorption/desorption, leaching, runoff, and plant uptake [8]. Phenoxy herbicide molecules are negatively charged and are therefore highly mobile at neutral pH. In groundwater, they are nonvolatile and persistent to hydrolysis, but they can be degraded biologically under both aerobic and anaerobic conditions. These herbicides demonstrate significantly greater persistence in temperate climates characterized by low winter temperatures and, in many regions, by depleted soil organic carbon content and acidic pH [7].
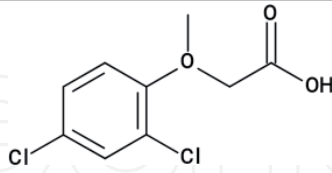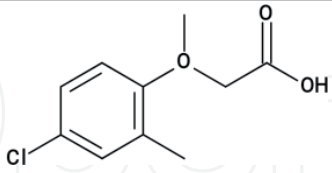
Properties	2,4-D	MCPA
Chemical structure		
IUPAC name	(2,4-Dichlorophenoxy)acetic acid	(4-Chloro-2-methylphenoxy)acetic acid
Molar mass (g/mol)	220.04	200.62
Solubility in H_2O (mg/L) [7]	450	720
pK_a [7]	2.73–2.87	3.73
K_{oc} [7]	20.56 mL/g	25–157 mL/g
logP [7]	2.50/2.58 ± 0.36	2.41/2.49 ± 0.27
t1/2 in water [9, 10]	1–14 days	15 days
t1/2 in soil [9, 10]	2–4 weeks	3–4 weeks

Table 1.
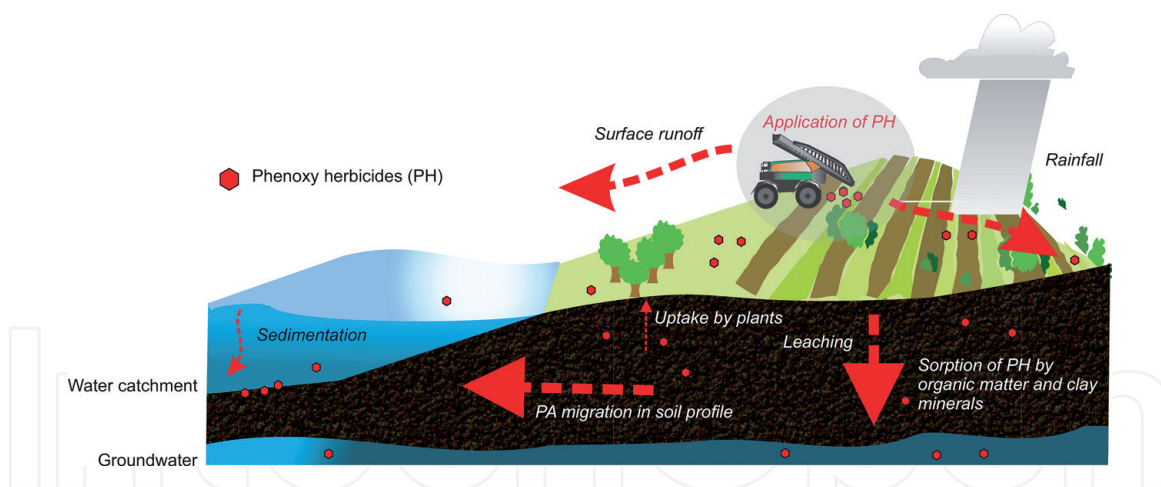*Physical and chemical properties of 2,4-D and MCPA.*

Figure 1.
Transport and transfer processes of phenoxy herbicides in the environment.

2. Phenoxy herbicides: potential contaminants of soil and water environments

Extensive use of phenoxy herbicides can threaten surface and groundwater ecosystems by promoting the contamination of soil matrices. The International Agency for Research on Cancer classifies phenoxy acids as "possibly carcinogenic to humans." Gupta et al. [11] report that 0.5 kg/ha is the optimal concentration of 2,4-D which avoids contamination of environmental matrices, with the effect of higher concentrations of 2,4-D on the environment being dependent on irrigation treatment. Hence, little is known of the distribution of phenoxy herbicides in the environment. Data from several sources have identified increased levels of 2,4-D and MCPA in the soil, ground-, surface, and drinking water (**Table 2**); for example, Ignatowicz and Struk-Sokołowska [12] note that the concentration of phenoxy herbicides in the Narew River (Poland) fluctuated seasonally from 0 to even 150 μg/L. The concentration of MCPA in the Parramatta River (Sydney Estuary, Australia) was 0.061 μg/L; however, its presence in river water was caused by increased runoff of storm water [13]. MCPA concentration has been found to be as high as 42.40 μg/L in the Rhone River (France) [14] and to be as little as 0.58 μg/L in Brejo of Cagarrão Stream (Portugal) [15]. The 2,4-D concentration has been found to vary from 1.678 μg/L in the water of McGregor Creek (Canada) [16] to 329.42 μg/L in water from a rice field [17]. By contrast, the maximum permissible concentration of pesticide residues in drinking water is 0.50 μg/L (Directive E98/83/EC). The data presented in **Table 2** and described above indicate that phenoxy herbicides should be considered as emerging contaminant especially in water resources.

Despite the diversified levels of phenoxy herbicides noted in worldwide environments (**Table 2**), it has to be underlined that these compounds can exert serious toxic effects on the sustainability of ecosystems, even at lower concentrations (e.g., 0.275 μg/L) (**Table 3**). According to recent research, predicted no effect concentration (PNEC) for aquatic organisms is 500 μg/L for 2,4-D and 0.022 μg/L for MCPA [20]; however, PNEC has not yet been determined for terrestrial organisms.

Because the mode of action of phenoxy herbicides mimics that of plant growth hormones, their application causes disturbances among a range of physiological processes [21]. 2,4-D inhibits root/hypocotyl elongation in *Sinapis arvensis* (wild mustard) and disrupts mesophyll cell structure in *Pisum sativum* (pea) [22, 23]. There is increasing concern that 2,4-D has negative influence on water ecosystems, leading to cellular deformation of green algae, such as *Ankistrodesmus falcatus* [24];

Compound	Concentration	Environmental matrices	Source
2,4-D	1.678 μg/L	Water from McGregor Creek (Canada)	[16]
2,4-D	103.99–329.42 μg/L	Water from rice field (Malaysia)	[14]
2,4-D	0.0052 mg/kg	Soil from cereal plantations (Poland)	[15]
2,4-D	0.513 μg/L	Lebo drain	[16]
MCPA, MCPP, 2,4-D	0–150 μg/L	Water from Narew River (Poland)	[12]
MCPA	0.0046 mg/kg	Soil from cereal plantations (Poland)	[15]
MCPA	0.08–42.40 μg/L	Water from Rhône River Delta (France)	[18]
MCPA	0.58 μg/L	Water from Brejo of Cagarrão Stream (Portugal)	[17]
MCPA	0.061 μg/L	Water from Parramatta River—Sydney Estuary (Australia)	[13]
MCPA	82.75–354.28 μg/L	Water from rice field (Malaysia)	[14]
MCPA	0.002–0.010 mg/kg	Soil from potato plantation (Poland)	[19]

Table 2.
Concentration of phenoxy acids observed in various environments.

Dose of phenoxy herbicide	Exposure time	Test organism	Effect on organism	Source
220.04 μg/L 2,4-D	2 days	*Sinapis arvensis* (wild mustard)	Inhibition of root and hypocotyl elongation	[22]
5.06 mg/L 2,4-D	72 hours	*Pisum sativum* (pea)	Severe disturbances in mesophyll cell structure and proliferation of vascular tissue in young leaves	[23]
10, 100, 500, 1000 μg/L MCPA	7 days	*Hydrilla verticillata* (waterthyme)	Disturbance of growth, anatomy, and physiology	[36]
IC50 1353.80 mg/L 2,4-D	96 hours	*Ankistrodesmus falcatus* (green microalgae)	External morphological alterations	[24]
IC50 71.20 mg/L 2,4-D	96 hours	*Microcystis aeruginosa* (toxigenic cyanobacteria)	Stimulation of the production of cyanotoxins	
LC50 66 mg/L 2,4-D	96 hours	*Cyprinus carpio* (common carp)	Behavioral changes	[26]
LC50 9.06 and 7.76 mg/L 2,4-D	96 and 168 hours	*Rhinella arenarum* (species of toad)	Reduced body size, delayed development, microcephaly, agenesis of gills, abnormal cellular proliferation processes	[27]
10–500 mg/L 2,4-D	1 hour	Human erythrocytes	Hemolysis	[29]
0.275, 2.75, and 27.5 μg/L 2,4-D and MCPA	30 minutes	Hepatic cells of *Metynnis roosevelti* (species of serrasalmid fish)	Damage of cellular metabolism and homeostasis; increased oxidative stress	[21]

Table 3.
The results of toxicological tests and effects of 2,4-D and MCPA on selected organisms.

malformations and behavioral changes to various fish, including *Cyprinus carpio* (common carp) and *Danio rerio* (zebrafish) [25, 26]; abnormal cellular proliferation in amphibians such as *Rhinella arenarum* (species of toad) [27]; and the development of nonviable embryos in invertebrates, such as *Biomphalaria glabrata* (species of freshwater snail) [28]. Sarikaya and Yilmaz [26] report that 2,4-D (66,000 μg/L) causes internal hemorrhage and behavioral changes in *C. carpio*.

Among animals, phenoxy herbicide application results in the inhibition of crucial enzymes in cell metabolism, including mitochondrial enzymes and those associated with DNA synthesis (**Table 3**). 2,4-D has also been found to induce erythrocyte lysis under laboratory conditions [29]. It is interesting to note that the intermediates formed during the degradation processes of 2,4-D, such as 2,4-dichlorophenol (2,4-DCP) and 3,5-dichlorocatechol (3,5-DCC), exhibit a strong ecotoxic effect on various organisms, including *N. tabacum* cells [30]; however, Taylor et al. [31] report that 2,4-DCP toxicity was found to be less phytotoxic than 2,4-D, under both in vitro and in vivo conditions, while 3,5-DCC exhibits higher toxicity than its parent compound [32].

Several studies have revealed that MCPA also can have a negative impact on the environment: MCPA application caused up to a 56% reduction in dehydrogenase, urease, and phosphatase activities and ergosterol content in soil [32]. In addition, this application leads to increased soil phytotoxicity to *Fagopyrum esculentum* var. Kora (buckwheat) and promoted stem deformation and leaf discoloration [33]. Mierzejewska et al. [34] note that a commercial product containing MCPA was highly toxic to the monocotyledon *Sorghum saccharatum* (sorghum) and dicotyledons *Lepidium sativum* (garden cress) and *Sinapis alba* (white mustard), inducing nearly 100% root growth inhibition. The authors also note that after 3 weeks of incubation at an ambient temperature, the high initial phytotoxicity was reduced to 3% for *L. sativum* and 34% for *S. alba* and that *S. saccharatum* demonstrated a 12% stimulation of root growth in comparison to uncontaminated control soil. The negative influence of MCPA on *L. sativum*, *S. alba*, and *S. saccharatum* growth was also confirmed by Urbaniak et al. [35]. Similarly to 2,4-D, MCPA causes also negative effects on freshwater organisms such as the freshwater crustaceans *Daphnia magna*, *Thamnocephalus platyurus*, and *Artemia franciscana* and alga *Selenastrum capricornutum* [36]. Both herbicides were found to induce the action of hepatic enzymes involved in detoxification and lipid peroxidation [21].

These studies emphasize the important role played by ecotoxicological approaches in evaluating the effect of chemical stressors observed in the ecosystem communities. Despite the relatively short half-life (**Table 1**) of 2,4-D and MCPA, their remnants can be transported and deposited extensively in the environment, and this can present a potential threat to the soil and water ecosystems as well as to human health. Therefore, there is a need to identify nature-based solutions such as bio-, phyto-, and rhizoremediation that can enhance the process of phenoxy herbicide elimination from the environment.

3. Phenoxy herbicides: removal using biological methods

One approach to removing phenoxy herbicides (2,4-D and MCPA) from soil is via degradation by the soil microbiota (biodegradation). This is achieved most effectively by bacteria harboring the appropriate functional genes, which are involved in the phenoxy herbicide degradation pathways (**Figure 2**). Alternatively, plants can be used to decontaminate sites, a process known as phytoremediation (**Figure 2**). Another promising approach, rhizoremediation, enhances the removal
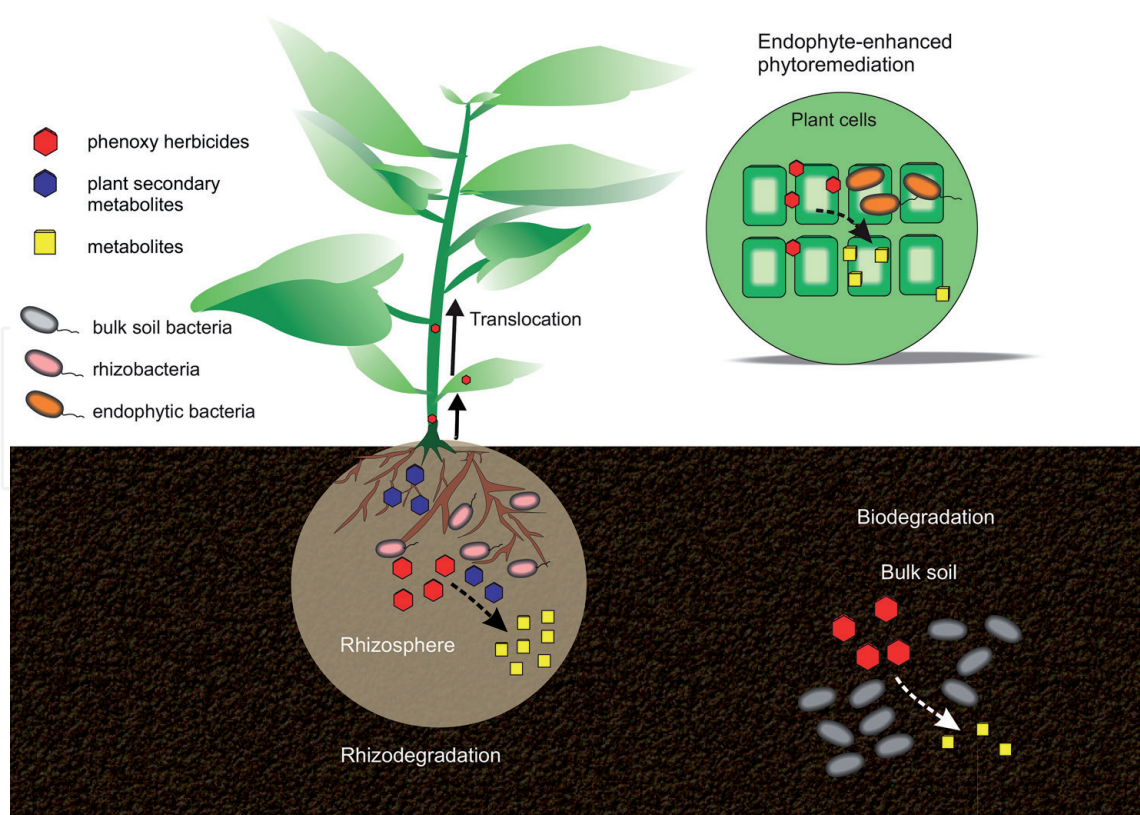
Figure 2.
The biological processes of phenoxy herbicide biodegradation mediated by soil, rhizospheric, and endophytic bacteria.

of such recalcitrant xenobiotics from the environment by exploiting the interactions between selected plants (able to grow under the presence of given xenobiotics such as phenoxy herbicides), root exudates (including plant secondary metabolites, PSMs), and microorganisms (**Figure 2**). The purpose of this section is to review the literature on established and potential biological methods of phenoxy herbicide removal from environmental matrices.

3.1 Bioremediation

Bioremediation is a method that uses microbiological processes to degrade or transform contaminants to less toxic or nontoxic forms. Biodegradation of organic contaminants occurs very slowly in bulk soil; therefore biostimulation and bioaugmentation methods are used to enhance the biologically driven removal of toxic compounds from environmental matrices. The effectiveness of biodegradation is dependent on several factors, among them the characteristics of the soil, the bioavailability of the contaminants, and their chemical properties.

An important way of phenoxy herbicide removal from soil is by the use of indigenous soil bacteria harboring desirable catabolic genes. The first step in the phenoxy herbicide biodegradation pathway is initiated by α-ketoglutarate-dependent dioxygenase, an enzyme encoded by *tfdA* or *tfdA*-like genes [37] located in the *tfdABCDEF* gene cluster [38].

In recent decades, increasingly rapid advances in the application of molecular analysis in environmental studies have helped identify the bacterial communities involved in phenoxy herbicide biodegradation (**Table 4**). The bacteria able to metabolize phenoxy herbicides have been classified into three groups as follows: according to their physiology, employed degrading enzymes, and evolutionary origin [39–41] (**Table 4**).

1. The first group consists of fast-growing copiotrophic bacteria belonging to β- and γ-proteobacteria harboring the *tfdA* gene (e.g., *Cupriavidus necator* JMP134, *Burkholderia* sp. strain RASC, and *Rhodoferax* sp. strain P230). This first group has been subdivided into four subclasses according to *tfdA* sequence: *tfdA* Class I, II, and III [42, 43] and *tfdA* α [38]. Class I is found in *Cupriavidus pinatubonensis*; Class II is less widely distributed, being found only in *Burkholderia* spp.; and Class III is found in *Comamonas acidovorans* [38]. *TfdA*α was first identified in *Bradyrhizobium* sp. [40]. However, *tfdA*α-encoded protein has been described as α-ketoglutarate-dependent 2,4-D dioxygenase with lower activity than JMP134 dioxygenase.

2. The second group consists of slow-growing oligotrophic bacteria belonging to α-proteobacteria, phylogenetically closely related to *Bradyrhizobium* sp. [41], which were isolated from pristine environments. In this group, the phenoxy herbicide degradative gene was also identified and classified as *tfdA*α. Its gene sequence shows 50–60% similarity to the Group I degrader *Cupriavidus necator* JMP134.

3. The third group consists of bacteria belonging to the α-proteobacteria harboring the *tfdA*α gene, with *Sphingomonas* being the key member [41]. The wide diversity displayed by *tfdA*-like genes can partly be attributed to the wide range of bacteria (α-, β-, and γ-proteobacteria) capable of degrading phenoxy acids in the environment. Due to the high degree of homology between strains, the *tfdA* genes have been selected as biomarkers of the capability of bacteria to metabolize 2,4-D and MCPA [37, 38], and they are frequently used in studies of phenoxy acid biodegradation.

Much of the current literature on phenoxy herbicide metabolic pathways pays particular attention to the degradation pathway of 2,4-D. One of the most extensively studied 2,4-D degraders is *Cupriavidus necator* JMP134, known to harbor the 80-kb pJP4 plasmid. pJP4 carries all of the structural and regulatory genes needed to convert phenoxy herbicides to 2-chloromaleylacetic acid [56]. The *tfdA* fragment is responsible for the conversion of 2,4-D to 2,4-DCP [57]. Subsequently, 2,4-DCP is hydrolyzed to 3,5-dichlorocatechol by 2,4-DCP hydroxylase, which is encoded by *tfdB*. 3,5-Dichlorocatechol is further degraded via a pathway encoded by *tfdCDEF*.

Far too little attention has been paid to the metabolism of MCPA. MCPA degradation takes place by the cleavage of an ether linkage, resulting in the formation of the major metabolite, 4-chloro-2-methylphenol (MCP), and acetic acid [47]. This process is preceded by the expression of the *tfdA* gene. Mierzejewska et al. [34] report that microorganisms demonstrating the presence of *tfdA*α and *tfdA* Class III genes in soil contaminated with a commercial product containing MCPA displayed biodegradation potential.

The bacteria carrying *cad* genes, which encode the non-heme iron oxygenase, also have the potential to degrade both herbicides. The *cadRABKC* gene cluster was first identified and characterized in strain *Bradyrhizobium* sp. HW3 which was isolated from pristine environment in Volcanoes National Park, Hawaii [39]. So far, however, there has been little research on the mode of action and exact function of *cad* genes. According to Kitagawa et al. [38], *cadA*, *cadB*, and *cadC* genes are responsible for multicomponent oxygenase production, whereas *cadR* is a transcriptional regulator gene, which regulates the transcription of *cadABKC* in the presence of 2,4-D or 4-chlorophenoxyacetic acid. The *cadA* gene products show structural and functional differences to the *tfdA* gene with regard to their substrate preferences. Both the *cadA* and *cadB* and the *tfdA* genes code for aromatic ring

Class	Strain	Origin	Studied compounds	Identified functional genes	Source
α-Proteobacteria	*Sphingomonas paucimobilis*	Soil from Michigan (USA)	2,4-D	—	[44]
	Sphingomonas agrestis 58–1	Soil from Fukuoka Prefecture (Japan)	2,4-D, MCPA	*cadA*, *cadB*	[45]
	Bradyrhizobium sp.; *Sphingomonas* sp.	Root nodules; pristine environments (Hawaii, central California, USA; southwestern Australia, southwestern Africa; central Chile; northern Saskatchewan, Canada; northwestern Russia); volcanic soil (National Park (Kipuka Keana Bihopa, Hawaii, USA)	2,4-D	*tfdAα*, *cadA*, and *cadB*	[38, 39, 41]
	Sphingomonas sp.	Sediment from an aquifer in Fladerne Creek (Denmark)	MCPA	*cadA* and *cadB*	[46, 47]
β-Proteobacteria	*Comamonas acidovorans* strain MCI	Herbicide-contaminated building rubble (Germany)	2,4-D and MCPA	*tfdB* and *tfdC* genes	[48]
	Variovorax paradoxus	Soil from the Dijon INRA experimental station (France)	2,4-D	*tfdA*, *tfdB*, and *tfdR*	[49]
	Delftia sp.	Polluted river in Buenos Aires (Argentina)	2,4-D	—	[50]
	Cupriavidus campinensis BJ71	2,4-D-enriched soils from wheat fields in Beijing exposed for 2,4-D for at least 10 years (China)	2,4-D	Class I *tfdA* gene	[51]
	Achromobacter sp. LZ35	Soil in a disused pesticide factory in Suzhou (China)	2,4-D and MCPA	*tfdA* and *tfdB*	[52]
	Halomonadaceae sp.	Alkali Lake site in Oregon (USA) contaminated with 2,4-D production wastes	2,4-D	*tfdA*	[53]
γ-Proteobacteria	*Pseudomonas pickettii*	Agricultural soil from Michigan (USA)	2,4-D	—	[54]
	Pseudomonas maltophilia	Wheat rhizosphere (laboratory experiment)	2,4-D	—	[55]

Table 4.
Bacteria degrading phenoxy herbicides isolated from pristine and contaminated environments.

hydroxylation dioxygenases (RHDO), which are widely distributed in a number of microorganisms and might be transferred through horizontal gene transfer [38]; *CadA-* and *cadB*-encoded proteins are involved in the same initial step of 2,4-D degradation; however, the enzyme subunits have a different mode of action to the ketoglutarate-dependent dioxygenase encoded by *tfdA*. *CadA* and *cadB* were mostly identified in bacteria belonging to Groups I and II of phenoxy herbicide degraders. The products of *cadA* gene expression are able to initiate the degradation of both MCPA and 2,4-D. Furthermore, the abundance of *cadA* gene stimulates MCPA degradation [47]. The *cadA* gene is also essential for 2,4-D conversion in pure cultures of α-proteobacteria [38, 45, 58], and the *cadB* gene is also thought to play a sole role in the phenoxy acid degradation; however, the exact role of the *cad* genes remains not fully understood. The two genes share ~50% identity with *tfdA*, and it has been found that *cadA*, *cadB*, and *tfdA* are expressed simultaneously during MCPA degradation. Interestingly, some bacteria harbor all three *cadA*, *cadB*, and *tfdA* genes, thereby displaying a dual system of degradative genes [47].

The microbial degradation metabolic pathway of phenoxy herbicides has been elaborated in recent years (**Figure 3**). The first step of this catabolic pathway is initiated by either the *tfdA* gene which encodes α-ketoglutarate-dependent dioxygenase or *cadAB* genes which encode subunits of non-heme iron oxygenase [47]. Although these enzymes use different modes of action, both catabolic proteins have been shown to perform the same initial step in phenoxy acid degradation, turning 2,4-D into 2,4-DCP and MCPA into MCP.

Until recently, there has been little interest in the stereospecific Fe-(II) α-ketoglutarate-dependent dioxygenases which are encoded by *rdpA* and *sdpA* genes. These enzymes are described in literature as the ones which can also initiate the first step of the MCPA and 2,4-D degradation pathway. They were identified in *Delftia acidovorans*, *Rhodoferax* sp., and *Sphingobium* sp. Although the proteins encoded by the *rdpA* and *sdpA* genes possess the highly conserved amino acid sequence motif of *tfdA*-encoded proteins, they share only 37% identity with the *tfdA* genes of *C. necator* JMP134 [60, 61].

In addition to the soil bacteria, soil microfauna can also profoundly affect the biodegradation of organic contaminants. An important example of this relationship is the activity of earthworms, which move through the soil, causing better aeration and increasing soil moisture. Hence, insofar as their activity can influence the profile of the microorganism communities in the soil, they can indirectly enhance the process of phenoxy herbicide aerobic bacterial degradation [61].

3.2 Phytoremediation

A steadily developing strategy for the in situ treatment of contaminated soils is phytoremediation. It is a cost-effective and environmentally friendly strategy that uses plants to transform or mineralize xenobiotics to less toxic or environmentally neutral compounds [62]. Plants play a crucial role in the development of soil structure and stabilization of fundamental soil ecosystem functions such as water flow [63]. They produce also an array of catabolic enzymes, which operate to protect the host organisms and detoxify xenobiotic compounds [64]. Therefore, phytoremediation not only contributes to the detoxification of the environmental matrices but also has a positive influence on the functioning of the entire ecosystem.

The process of contaminant absorption by plants depends on several factors, including regional climate, soil type, and the nature of the pollutant [65]. The selection of an appropriate plant species and cultivar is critical for effective removal of a given contaminant from soil [66, 67]. This choice of phytoremediation candidate should
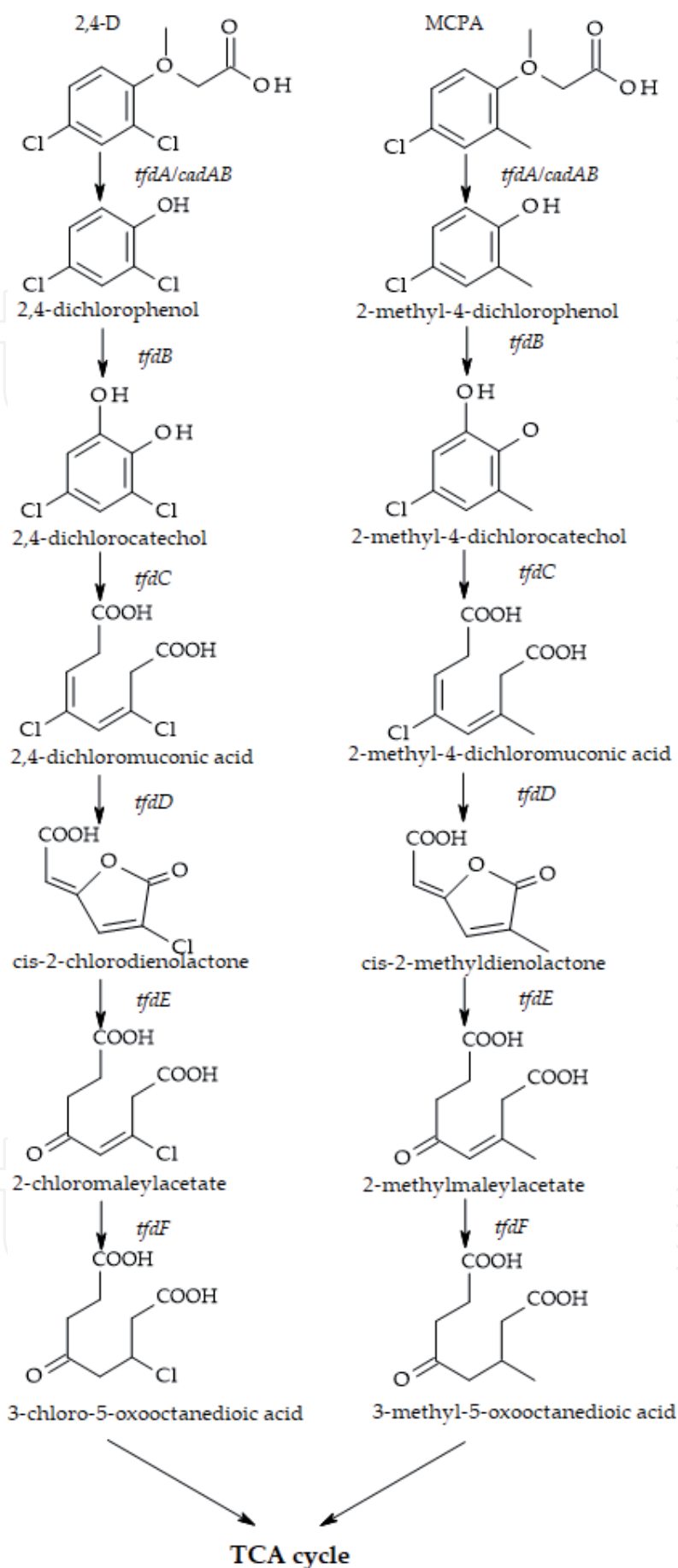
Figure 3.
Pathways of microbial degradation of 2,4-D and MCPA proposed by Pieper et al. [59]; in the picture there are indicated functional genes which encode catabolic enzymes as follows: tfdA, α-ketoglutarate-dependent dioxygenase; cadAB, subunits of non-heme iron oxygenase; tfdB, chlorophenol hydroxylase; tfdC, catechol 1,2-dioxygenase; tfdD, dichloromuconate cycloisomerase; tfdE, carboxymethylene butenolidase; tfdF, maleylacetate reductase.

particularly take into account plant growth rate, high biomass production, capacity for pollutant accumulation, and tolerance to higher xenobiotic concentrations [67].

In terms of phenoxy herbicide removal, there has been little investigation of the plant-mediated removal of 2,4-D and/or MCPA. For example, Ramborger et al. [68] evaluated the phytoremediation potential of *Plectranthus neochilus* (tea) exposed to the commercial pesticide containing 2,4-D (Aminol) in soil and water. The removal rate for 2,4-D reached 49% during 60 days, and the herbicide was not detected in plant leaves. Despite the fact that the phytoremediation potential of *P. neochilus* in soil was not sufficient, the plant exhibited satisfactory resistance to herbicide application. Moreover, the presence of phenolic compounds (e.g., ferulic and coumaric acid) in tea tissues indicated the ability of these plants to provide defense mechanisms against 2,4-D. The mechanism of the herbicide in plant begins by affecting the plasma membrane properties, subsequently leading to poor performance of mitochondria and peroxisomes [69]. In consequence, it stimulates the overexpression of abscisic acid (ABA) and ethylene biosynthesis genes, leading to significant changes of cellular redox potential by the production of reactive oxygen species (ROS) [70]. The occurrence of ROS leads to the production of phenolic compounds, i.e., ferulic acid and coumaric acid, which are responsible for the antioxidant self-defense mechanism of the plant against the herbicide. These phenolic compounds were found in higher concentrations only in plants that were exposed to 2,4-D and not in the controls.

3.3 Rhizoremediation

As mentioned above, plants play a key role in soil ecosystems by stabilizing the soil structure and by serving as primary sources of organic matter and energy which stimulate soil microbial activity [63]. Despite this, they are not the only contributors in the efficient phytoremediation of organic contaminants. Due to existing interactions between plant roots, root exudates, soil, and microorganisms, it has been proposed that the most effective method for the remediation of contaminated soil may be microbe-assisted phytoremediation (rhizoremediation).

Rhizoremediation is a naturally occurring process within the plant root zone (rhizosphere), where the growth of microorganisms and their degradative activity are stimulated by root exudates enriched by plant secondary metabolites (PSMs). Plant-derived compounds can [1] serve as primary substrates in cometabolism and provide energy for microbial growth [2], act as inducers of degradative enzymes due to their structural similarities to xenobiotics, and [3] enhance the degree of contamination removal by increasing pollutant bioavailability in soil [71].

The effectiveness of rhizospheral biodegradation depends also on the potential of the microorganisms inhabiting the rhizosphere to adapt to pollutant concentrations [72]. For effective degradation of contaminants to take place, a wide range of plants and bacterial traits is needed, involving the orchestrated interaction of a multitude of genes and enzymes. Rhizoremediation can therefore be optimized by selecting suitable plant-microbe sets, which can be achieved by combining plant and plant growth-promoting rhizobacteria (PGPR) [73] and/or microbes capable of contaminant degradation [74]. PGPR can improve phytoremediation efficiency by enhancing plant tolerance to various environmental stresses, promoting root growth and improving plant growth and health. In turn, some rhizospheral microorganisms can directly use their own degradative capabilities to metabolize organic pollutants [74, 75]. A study of rhizosphere-enhanced biodegradation of 2,4-D by Boyle et al. [76] found a significant difference in the mineralization of 2,4-D between monocot rhizosphere soils, dicot rhizosphere soils, and non-rhizosphere soils, with greater microbial activity being observed in monocot

rhizosphere soil than in dicot rhizosphere soil or bulk soil. Therefore, both the soil and plant species determine the mineralization of tested contaminant. According to Shaw and Burns [77], the amendment of soil with 2,4-D increased the number of rhizospheric bacteria degrading 2,4-D in *Trifolium pratense* (red clover). Germaine et al. [78] also note the abundance of 2,4-D degraders in the stem and leaves of pea plant and that, under exposure to phenoxy herbicide, pea plants developed a stubby root system.

Furthermore, it has been hypothesized that PSMs may have a profound impact on the biodegradation of xenobiotics by providing the energy for microorganisms to carry out cometabolism; in this case, the xenobiotic is degraded as a secondary substrate [45, 71–73]. PSMs can be used as a primary source of carbon for bacterial communities to support their growth and stimulate the expression of desirable genes involved in the catabolic pathway of given xenobiotic. This is evident in the case of biphenyl, naringin, coumarin, myricetin, and l-carvone, which stimulate the activity of polychlorinated biphenyl (PCB)-degrading bacteria such as *A. eutrophus*, *Corynebacterium* sp., *P. putida* [79], and *Arthrobacter* sp. strain B1B [80]. Another example of PSM-stimulated PCB biodegradation was identified in mulberry (*Morus* sp.). In this case, the PSMs morusin, morusinol, and kuwanon C have been found to support the growth of the PCB-degrading bacterium *Burkholderia* sp. LB400 [81]. Likewise, the PSM (cumene) stimulates the activity of TCE-degrading *R. gordonia* bacteria [85]. According to Yi et al. [82], salicylic and linoleic acids, excreted by *Raphanus sativus*, enhanced the bioavailability of polycyclic aromatic hydrocarbons (PAHs) and increased the effectivity of their removal form soil. According to Ely and Smets [83], PAH biodegradation is stimulated by the presence of phenolic compounds, flavonoids, and gibberellic acid. Compounds such as acetophenone, phenethyl alcohol, p-hydroxybenzoic acid, and trans-cinnamic acid enhance the biotransformation of cis-1,2-dichloroethylene [84].

In addition, it has been hypothesized that PSMs may also induce the detoxification mechanisms taking place in bacterial cells [85, 86]. The expression of functional genes in bacteria is essential for the successful bioremediation of xenobiotics and can be stimulated by PSMs in different ways. However, very little information is given in the literature regarding the influence of PSMs on the induction of genes involved in catabolic pathways. Siciliano et al. [87] report greater induction of catabolic genes (*ndoB*, *alkB*, *xylE*) involved in the degradation of naphthalene in the rhizosphere soil of *Festuca arundinacea* (tall fescue) than in unplanted soil. Salicylate has been reported to have an upregulating effect on the expression of *bphA*, which encodes biphenyl dioxygenase in the PCB degrader *Pseudomonas* sp. Cam-1 [88]. The presence of salicylic acid was found to enhance the expression of the *bphA* gene in *R. eutropha* H850 and *P. fluorescens* P2W [89].

In addition, it has been hypothesized that the structural similarity between selected xenobiotics and PSMs may have a profound impact on the biodegradation of given, structurally related xenobiotic [71, 85]. For example, Urbaniak et al. [35] demonstrated the effect of a PSM, syringic acid, on the enhanced removal of structurally similar herbicide, MCPA, by indigenous soil bacteria, with greater MCPA depletion being achieved in samples enriched with PSM. The molecular analysis revealed ubiquitous enrichment of the samples with *Rhodoferax* spp., *Achromobacter* spp., *Burkholderia* spp., and *Cupriavidus* spp., which are commonly known as MCPA degraders. Also, a study by McLoughlin et al. [89] found the PSMs limonene and α-pinene to enhance 2,4-DCP degradation, but only following pre-exposure to both 2,4-DCP and monoterpene, with total 2,4-DCP mineralization extents of up to 71%.

Taking into account the abovementioned aspects, rhizoremediation can serve as a potential tool for phenoxy herbicide removal from soil ecosystems. However, to date, most studies have focused solely on the phyto- or biodegradation properties

of plants or bacteria [71]. Consequently only limited data is available in terms of the impact of rhizoremediation on phenoxy herbicide removal from soil.

3.4 Endophyte-enhanced phytoremediation

Endophytic bacteria that reside inside plant tissues are also known to play a crucial role in the remediation of organic compounds. Plant-associated bacteria can enhance plant growth and degrade organic contaminants such as trichloroethylene and hydrocarbons [90]. The activity of endophytic bacteria can mitigate and improve plant conditions in stressful environments (such as contaminated soils). Field studies by Eevers et al. [91] showed that zucchini (*Cucurbita pepo*) plants inoculated with a consortium of three plant growth-promoting endophytic strains demonstrated an increased concentration of dichloro-bis(p-chlorophenyl)ethylene (DDE) in the aerial parts. The amount of DDE accumulated in *C. pepo* per growing season was significantly higher for inoculated plants. Thus such an approach might be promising for phytoremediation applications.

It has also been found that application of 2,4-D (1.42, 2.84, and 5.68 mg a.i./g soil) had a negative effect on the physio-morphological parameters of aerobic rice

Method	Compound	Initial concentration of compound used in an experiment	Duration of an experiment (days)	Removal of phenoxy-acetic acid (%)	Comments	Source
Bioremediation	2,4-D and MCPA	0.09 mmol/kg of soil	118	60	Activity of bulk soil microbial population from various soil samples	[93]
	2,4-D	1.8 kg/ha	10	45–48	Activity of bulk soil microbial population from clay and loamy soil samples	[94]
Phytoremediation and rhizoremediation	2,4-D	11.42 kg/ha	20	49	Use of *P. neochilus* for phytoremediation	[76]
	2,4-D 2,4-DCP	$1.22 \ 10^{-3} \ \mu m$ $1.19 \ 10^{-3} \ \mu mol$	66	~60	Activity of rhizospheric soil bacteria derived from monocots	[68]
Endophyte-enhanced phytoremediation	2,4-D	47–360 mg/kg of soil	53	93–100	The inoculation of *P. sativum* by endophytic bacteria *P. putida* VM1450	

Table 5.
Biological remediation methods and % average removal of phenoxy herbicides from soil matrices.

(*Oryza sativa*) and reduced the number of plant endophytes [92]; however, inoculation of seeds with the endophytic bacteria strain *Stenotrophomonas maltophilia* improved plant characteristics under herbicide-stressed soils. *S. maltophilia* has previously been described as a plant growth-promoting endophytic strain with the ability to produce auxins and siderophores [92].

Bacterial endophyte-enhanced phytoremediation was also studied by Germaine et al. [78] on the example of *P. sativum*: plants were inoculated with genetically tagged endophytic bacteria, which naturally possess the ability to biodegrade 2,4-D. The inoculated plants not only displayed more efficient herbicide removal but also demonstrated a lack of 2,4-D accumulation in their aerial parts. Additionally the endophytic strain protected the pea plant from the toxic effects of 2,4-D, resulting in a greater increase of plant biomass and thus greater 2,4-D transportation to the aboveground parts of the plant from the soil.

Table 5 compares the presented biological methods of remediation of soils contaminated with phenoxy herbicides. It illustrates the differences of the removal of phenoxy herbicides from soil. It is apparent from this table that the most efficient method of contaminant removal is endophyte-assisted phytoremediation; however, more research on this topic needs to be undertaken before the association between role of symbiotic microorganisms and plants in removal of contaminants from environmental matrices is more clearly understood.

4. Conclusions

Uncontrolled use of phenoxy herbicides (2,4-D and MCPA) in the agricultural and gardening sector can result in their dispersal in soil and water ecosystems, which can significantly disturb the sustainability of the environment and increase its ecotoxicity level. Although their persistence in soil is limited due to their chemical characteristics, they can be transported and accumulated in water ecosystems through runoff and leaching. According to recent reports, phenoxy herbicides are especially toxic for plants, freshwater crustaceans, and amphibians; hence there is a growing need to limit the release of phenoxy acids in natural environments.

Taking into account the abovementioned aspects, the integration of bio-, phyto-, and rhizoremediation can serve as a potential tool for phenoxy herbicide removal from soil ecosystems. The ability of bacteria to metabolize phenoxy herbicides has been extensively studied over the last decades. However, to date, only limited data is available in terms of the impact of phyto- and rhizoremediation on phenoxy herbicide removal from soil. What is not yet clear is the impact of PSMs on the degradation of phenoxy herbicides. The similarity of the chemical structure of chosen PSMs and xenobiotics can be reflected in the xenobiotic degradation rates, e.g., the presence and induction of degradative genes and production of degradative enzymes, and the composition of microbial populations. To date, little evidence has been found associating the removal of phenoxy herbicides using both plants and microorganisms. However, the abovementioned research serves as a base for future studies on their application for the improvement of soil quality.

Considering the above, the chapter describes an interdisciplinary approach to tackling the problem of environmental phenoxy acid herbicide contamination through integrating available literature data on the physicochemical properties of 2,4-D and MCPA, as well as their levels in the environment and toxicity to the organisms from different trophic levels. It also outlines possible methods for their removal using nature-based techniques such as bio-, phyto-, and rhizoremediation.

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Conflict of interest

There is no conflict of interest.

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