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# Biodegradation of PCDDs/PCDFs and PCBs

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Additional information is available at the end of the chapter

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## 1. Introduction

As a consequence of the rapid development of modern society during the 20<sup>th</sup> century, a significant amount of organic chemicals has been dispersed into the environment. Many of them have been used as pesticides, insecticides, defoliant and industrial chemicals or produced as undesirable industrial by-products. A large amount of them show several metabolic and toxic activities including mutagenic, immunotoxic and carcinogenic effects. From this group of substances, the organochlorine compounds include polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs), which have received the most attention according to their persistence in the environment, bioaccumulation and hazard for biota [1].

### PCDDs/PCDFs

PCDDs and PCDFs are a group of organic chemicals that contain 75 structurally related individual congeners widely distributed in the environment. They were present on Earth for a long time before humans, as they are formed as a result of forest fires and volcanic explosions. They are also manufactured as unwanted by-products in a range of processes, such as municipal waste incineration, metal smelting, chlorine bleaching in the pulp and paper industry, and vehicular emissions. Such a variety of PCDD/PCDF sources causes their widespread occurrence in the environment. They have been detected in soil, surface water, sediments, plants and animal tissue in all regions of the Earth [2,3].

Chlorinated dioxin's precursor is dibenzo-*p*-dioxin, which consists of two benzene rings bridged by oxygen [4-8] (Fig. 1).

Polychlorinated dibenzofurans are similar to polychlorinated dibenzo-*p*-dioxins, in terms of chemical structure and biological activity (Fig. 2).



**Figure 1.** The structural formula of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [9, changed].



**Figure 2.** The structural formula 2,3,7,8-tetrachlorodibenzofuran (9, changed).

The physical and chemical properties of toxic congeners of PCDD and PCDF are depicted in Table 1 and 2, respectively.

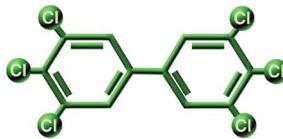
Compound	Melting point (25°C)	Solubility in water in mg/l (25°C)	Vapour pressure (Pa) in 25°C	Log $K_{ow}$
2,3,7,8-TCDD	305-306	$1.93 \times 10^{-3}$	$2.0 \times 10^{-7}$	6.8
1,2,3,7,8-PeCDD	240-241	$1.93 \times 10^{-3}$	$5.8 \times 10^{-8}$	6.64
1,2,3,4,7,8-HxCDD	273-275	$4.42 \times 10^{-6}$	$5.1 \times 10^{-9}$	7.8
1,2,3,6,7,8-HxCDD	283-286	$4.42 \times 10^{-6}$	$4.8 \times 10^{-9}$	7.8
1,2,3,7,8,9-HxCDD	243-244	$4.42 \times 10^{-6}$	$6.5 \times 10^{-9}$	7.8
1,2,3,4,6,7,8-HpCDD	264-265	$2.4 \times 10^{-6}$	$7.5 \times 10^{-10}$	8.0
OCDD	325-326	$0.75 \times 10^{-7}$	$1.1 \times 10^{6.8}$	8.2

**Table 1.** Physical and chemical properties of PCDDs [10, changed].

PCBs, in turn, due to their stable properties such as low dielectric constant, chemical inertness, non-flammability, high heat capacity, high electrical resistivity and low acute toxicity, were found to be ideal for industrial applications and thus were produced and used in many countries including the United States, Russia, Japan, France and Czechoslovakia. Global PCBs use is estimated to be 1.2 to 1.5 million tonnes. Although the production and use of PCBs was banned almost all over the world more than 30 years ago due to their toxic effects on humans and biota, they are still detected in many ecosystem compartments [11-14]. The PCB molecule consists of two phenyl rings, in which the chlorine atoms are substituted in place of hydrogen atoms. Theoretically, there could be 209 individual PCB congeners (Fig. 3).

Compound	Melting point (25°C)	Solubility in water in mg/l (22.7°C)	Vapour pressure (Pa) in 25°C	Log K <sub>ow</sub>
2,3,7,8-TCDF	227-228	4.19 x 10 <sup>-4</sup>	2.0 x 10 <sup>-6</sup>	6.53
1,2,3,7,8-PeCDF	225-227	4.19 x 10 <sup>-4</sup>	2.3 x 10 <sup>-7</sup>	6.79
2,3,4,7,8-PeCDF	196-196.5	2.36 x 10 <sup>-4</sup>	3.5 x 10 <sup>-7</sup>	6.92
1,2,3,4,7,8-HxCDF	225.5-226.5	8.25 x 10 <sup>-6</sup>	3.2 x 10 <sup>-8</sup>	6.92
1,2,3,6,7,8-HxCDF	232-234	1.77 x 10 <sup>-6</sup>	2.9 x 10 <sup>-8</sup>	6.92
1,2,3,7,8,9-HxCDF	246-249	1.77 x 10 <sup>-6</sup>	2.4 x 10 <sup>-8</sup>	6.92
2,3,4,6,7,8-HxCDF	239-240	1.77 x 10 <sup>-6</sup>	2.6 x 10 <sup>-8</sup>	6.92
1,2,3,4,6,7,8-HpCDF	236-237	1.35 x 10 <sup>-6</sup>	4.7 x 10 <sup>-9</sup>	7.92
1,2,3,4,7,8,9-HpCDD	221-223	1.35 x 10 <sup>-6</sup>	6.2 x 10 <sup>-9</sup>	7.92
OCDF	258-260	1.16 x 10 <sup>-6</sup> (in 25°C)	5 x 10 <sup>-9</sup>	8.78

**Table 2.** Physical and chemical properties of PCDFs [10, changed].



**Figure 3.** The structural formula of 2,2', 3,3', 4,4'-hexachlorobiphenyl [9, changed].

PCBs have been produced under several trade names, e.g., Clophen (Bayer, Germany), Aroclor (Monsanto, USA), Kanechlor (Kanegafuchi, Japan), Santothrem (Mitsubishi, Japan), Phenoclor and Pyralene (Prodolec, France) (Table 3).

Apiolio	Diaclor	No-Flamol
Areclor	Duconol	Pydraul
Aroclor	Dykanol	Pyralene
Arubren	Elemex	Pyranol
Asbestol	Euracel	Pyroclor
Askarel	Fenchlor	Phenoclor
Bakola	Hivar	Saf-T-Khul
Biclор	Hydol	Santotherm
Chlorextol	Inclor	Santovac
Chlorinol	Iterteen	Siclonyl
Chlorphen	Kennechlor	Solvол
Clophen	Montar	Sovol
Delor	Nepolin	Therminol

**Table 3.** Major trade names of PCBs [15].

Commercial PCBs are complex mixtures of 30–60 congeners, which are the major PCB components of most environmental extracts. Each individual compound shows a unique combination of physico-chemical and biological properties dependent on the degree of chlorination (Table 4).

Aroclor compound	Water solubility (mg/l) 25°C	Vapour pressure 25°C	Density 25°C [g/cm <sup>3</sup> ]	Appearance	Boiling point [°C]
Aroclor 1016	0.4200	4.0×10 <sup>-4</sup>	1.33	Clear oil	325–356
Aroclor 1221	0.5900	6.7×10 <sup>-3</sup>	1.15	Clear oil	275–320
Aroclor 1232	0.4500	4.1×10 <sup>-3</sup>	1.24	Clear oil	290–325
Aroclor 1242	0.2400	4.1×10 <sup>-3</sup>	1.35	Clear oil	325–366
Aroclor 1248	0.0540	4.9×10 <sup>-4</sup>	1.41	Clear oil	340–375
Aroclor 1254	0.0210	7.7×10 <sup>-5</sup>	1.50	Light, yellow, viscous oil	365–390
Aroclor 1260	0.0027	4.0×10 <sup>-5</sup>	1.58	Light, yellow, viscous oil	385–420

**Table 4.** Physical and chemical properties of selected Aroclors [15, after 16].

Currently, many countries impose strict controls on the use and release of PCDDs/PCDFs and PCBs. As a result their input into the environment has decreased significantly. Nevertheless, their release from contaminated sites and their redistribution on a global scale is still observed [17–18]. Their slow decomposition in the environment and the hazards they pose for living organisms makes PCDDs/PCDFs and PCBs large-scale environmental degraders, especially because their toxicity can be further enhanced by their ability to accumulate in the soil and sediments and their bioaccumulation and biomagnification within aquatic and land food chains (Fig. 4).

It should also be underlined that PCDDs/PCDFs and PCBs also pose a risk to human health. They have been shown to produce toxic responses similar to those caused by 2,3,7,8-TCDD, the most potent congener within this group. Studies on animals demonstrate that PCDDs/PCDFs and PCBs are implicated in mutagenic and carcinogenic effects such as liver damage, malignant melanoma and preneoplastic and neoplastic changes [1, 19]. Other manifestations related to PCDDs/PCDFs and PCBs are gastrointestinal (gastric hyperplasia, ulceration, necrosis), respiratory (chronic bronchitis and coughs), dermal (chloracne, oedema, alopecia, hyperkeratosis of epithelium), neurotoxic (impaired behavioural responses, depressed motor activity, developmental deficits, numbness) and immunotoxic (lymphoid tissue atrophy, leukocyte and lymphocyte reduction, suppressed antibody responses), hepatotoxic (hepatomegaly, hyperplasia of the bile duct, necrosis, fatty degeneration, porphyria) and reproductive problems (decreased sperm motility and number, increased miscarriages, decreased survival and mating success) [1, 19].

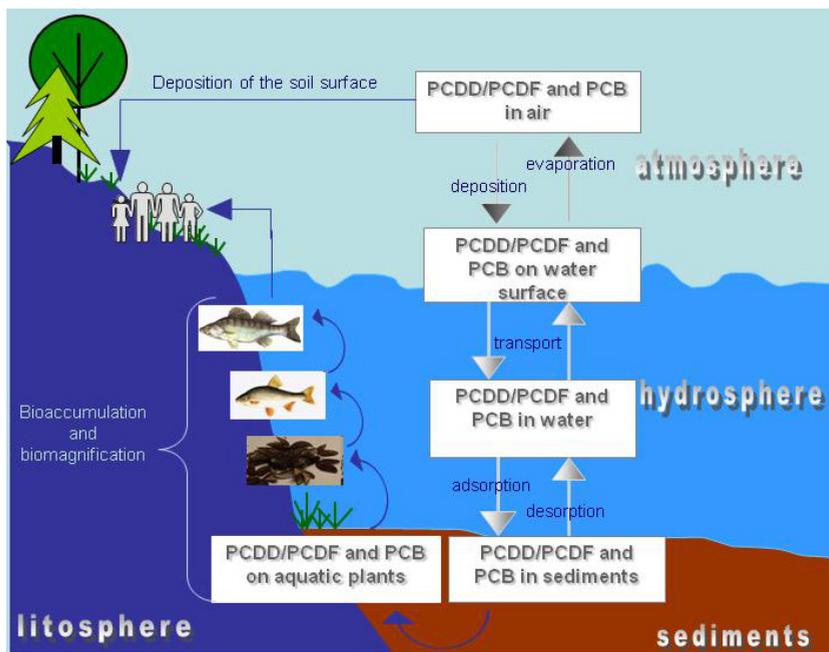


Figure 4. Transport and circulation of PCDDs/PCDFs and PCBs in the environment.

## 2. Microbiological transformation of PCDDs/PCDFs and PCBs

The degradation of PCDDs/PCDFs and PCBs is classified into two sections: biological transformation by microorganism activity and physico-chemical transformation.

The first group includes anaerobic, aerobic and sequential anaerobic-aerobic transformation. The latter can be classified into photochemical and thermal degradation.

Microbiological transformation depends on enzymes produced by microorganisms which enable modification of toxic compounds into less toxic forms. Biological degradation can carry on as mineralization when microorganisms use the organic compound as a source of carbon and energy, or as co-metabolism where microorganisms need other sources of carbon and energy and the transformation of pollutants occurs as a concurrent process. Products of this process can be further mineralized, otherwise incomplete degradation occurs, leading to the formation and accumulation of more toxic metabolites than parent substrates.

The effectiveness of degradation rates varies depending on the conditions present in the environment and comprises: 1) input of pollutants, 2) physical parameters (oxygen content, temperature, light intensity, pH, conductivity) and 3) biological parameters (presence of microorganisms able to degrade a given pollutant and the availability of carbon and/or other sources of energy). All of the above variables determine the rate of biological and physical transformation of analysed compounds.

## 2.1. Aerobic conditions

### Bacterial cometabolism

Aerobic transformation occurs in environments that are rich in oxygen and involves the use of microbial molecules, such as mono- and dichlorinated PCDDs/PCDFs and PCBs, as a source of carbon and energy. It should be noted that in about 90% of cases, the process takes place as co-metabolism, which means that the microorganisms need an additional source of carbon apart from PCDDs/PCDFs or PCBs.

Data from the literature confirms the aerobic biodegradation of PCDD/PCDF and PCB compounds and the rate of this process increases with the reduction of PCDD/PCDF and PCB chlorination [20-23]. Thus, for example, molecules containing five or more chlorine atoms are not susceptible to the effects of aerobic microorganisms.

### PCDDs/PCDFs

In the case of PCDDs and PCDFs the research conducted over the last 30 years has widely described their aerobic biodegradation [19-22, 24]. Worldwide studies have demonstrated that many isolated strains of bacteria, such as *Rhodococcus opacus* SAO101, *Beijerinckia* sp. B8/36, *Pseudomonas veronii* PH-03, *Pseudomonas* sp. HH69, CA10, EE41, *Bacillus megaterium* AL4V, *Sphingomonas* sp. RWI and HL7, are capable of the biodegradation of slightly chlorinated PCDDs/PCDFs under aerobic conditions [21, 24-29]. To increase the rate of aerobic biodegradation of PCDDs/PCDFs and PCBs an additional source of carbon, for example a small amount of un-substituted PCDD or biphenyls [20], carbazole [30], o-dichlorobenzene [25] or benzoic acid or 3-methoxybenzoic [30] can be used.

### PCBs

The first data on the aerobic degradation of PCBs was reported by Ahmed and Focht [31] in 1973 and the respective study was devoted to the degradation of biphenyl and monochlorobiphenyl to chlorobenzoic acid by two species of *Achromobacter*. Furukawa et al. [32] demonstrated that a species of *Acinetobacter* and *Alcaligenes* can rapidly adsorb 2,5,2' trichlorobiphenyl onto the cell surface, then metabolize and release metabolic compounds from the cell. Since then numerous investigations have focused on the occurrence and distribution of PCB-degrading microorganisms and their capability to biodegrade PCBs. For example, Clark et al. [33] reported that *Alcaligenes denitrificans* and *A. odorans* can degrade Aroclor 1242 (a mixture of PCB containing 42% chlorine) by co-metabolism. A study by Novakova et al. [34] showed the results of the degradation of Delor 103 by *Pseudomonas* sp. P2 and *Alcaligenes eutropha*. Optimal PCB degradation was obtained by the addition of biphenyl, saccharose, agar or an amino acid mixture as the source of carbon. A reduction of degradation efficiency was observed by the addition of glycerol or pyruvate. To completely degrade PCBs by aerobic bacteria, various microbial strains with specific congener preferences are required.

### Bacterial mineralization

According to data described by Field and Sierra-Alvarez [35] there are few well documented examples of chlorinated PCDDs/PCDFs and PCBs serving as the sole source of carbon and

energy for pure bacterial strains. This is shown by the research of Hong et al. [28] wherein the *Pseudomonas veronii* PH-03 has been used to utilize 1-CDD and 2-CDD growing on aliphatic acids generated from ring cleavage. The mentioned strain of *Pseudomonas veronii* accumulated the dead products 3-chlorocatechol and 4-chlorocatechol from the chlorinated rings. Similar results were also obtained by Arfmann et al. [36] by using a *Sphingomonas* sp. strain RW1 growing on 4CDF. The substrate of carbon and energy was a 5-carbon aliphatic acid and a 2-hydroxypenta-2,4-dienoate released from the ring cleavage and the dead-end products were 3-chlorosalicylic acid.

The complete mineralization of PCDDs/PCDFs was also achieved by using co-cultures including a PCDD/PCDF-degrader and a 3-chlorosalicylic acid-degrader. For example, a study by Wittich et al. [37] showed that use of *Sphingomonas* sp. RW16 and *Pseudomonas* sp. RW10 enabled the complete degradation of 2-CDF and 3CDF. The co-culture mixture combined with *Sphingomonas* sp. RW1 and *Burkholderia* sp. JWS was shown to completely degrade 4-CDF [36]. The above research demonstrates that *Sphingomonas* sp. RW16 and *Sphingomonas* sp. RW1 were capable of degrading the CDF and the *Pseudomonas* sp. RW10. *Burkholderia* sp. JWS utilized the 3-chlorosalicylic acid as the released as dead-end product.

### **Fungal cometabolism**

It should also be mentioned that fungi, similarly to bacteria, are capable of PCDD/PCDF degradation in aerobic conditions, in both mineralization and the co-metabolism process.

Fungi use enzymes (lignin peroxidase or manganese peroxidase) to oxidise the molecule of the compound. The first described case of use of the fungal biodegradation is the work of Bumpus et al. [38], in which the authors documented the mineralization of [<sup>14</sup>C] 2,3,7,8-TCDD to <sup>14</sup>CO<sub>2</sub> within 30 days by the fungi of *Phanerochaete chrysosporium*. *P. chrysosporium* has also been successfully used to degrade 2,7-DCDD [39].

The biodegradation activity of fungi is not limited to less chlorinated congeners. There is evidence that *P. chrysosporium* is able to remove 34% and 48% of a mixture of congeners containing from 5 to 8 chlorine atoms in the molecule during 7 and 14 days [40].

## **2.2. Anaerobic conditions**

Anaerobic microorganisms are well adapted to pollutants with a high carbon concentration due to the diffusional limitation of oxygen. Anaerobic transformations of PCDDs/PCDFs and PCBs include reductive dehalogenation using PCDDs/PCDFs and PCBs as electron acceptors. During this process a substituent chlorine atom is replaced with a hydrogen atom.

Reductive dehalogenation occurs in soils and sediments, where different microorganisms possessing dehalogenation enzymes responsible for dechlorination and dehalogenation processes exist. The rate, extent and route of dechlorination are dependent on environmental factors, such as carbon availability, electron donors, presence of electron acceptors other than PCDDs/PCDFs and PCBs, temperature and pH. All of these factors influence the composition of a microorganism's community and their activity.

### **PCDDs/PCDFs**

The first evidence of degradation of PCDDs/PCDFs under anaerobic conditions was obtained by spiking sediment microcosms with highly chlorinated congeners of HpCDD, HxCDD and PeCDD [40]. The rate of removal of those compounds in biologically active sediments was from 19% to 56% higher in comparison to heat-killed sediments. The products of such biodegradation processes were TCDD and TCDF congeners [40, 41]. The main microorganisms capable of efficient degradation of these compounds were mainly bacteria of the genus *Dehalococcoides* [43-45]. Experiments with the use of OCDD (8 chlorine atoms) at a concentration of 5.3 ml/L applied into sediment microcosms, showed that after 7 months the congener was distributed into forms that contain only 1 to 3 chlorine atoms [46-47].

### PCBs

The first evidence of anaerobic degradation of PCBs was reported based on the observed modification of Hudson River and Silver Lakes sediments contaminated by commercially produced PCBs. The increase of low-chlorinated PCBs in comparison to the high-chlorinated congeners was consistent with reductive dechlorination [48]. Furukawa et al. [49] demonstrated that species of *Acinetobacter* and *Alcaligenes* may rapidly adsorb 2,5,2'-trichlorobiphenyl onto the cell surface and then metabolise and release metabolic compounds from the cell. From that time many of investigations were devoted to the occurrence and distribution of PCB-degrading microorganisms and their capability to biodegrade PCBs.

Master et al. [48] showed that many commercial PCB mixtures can be reductively dechlorinated under anaerobic conditions, for example, Aroclor was dechlorinated at rates of 3 µg Cl/g of sediment per week. The dechlorination occurs at temperatures of 12°C and PCB concentrations of 100–1000ppm [49]. Fava et al. [50] described the degradation of Aroclor 1242 by three strains: *Comamonas testosteroni*, *Rhodococcus rhodochrus* and *Pseudomonas putida* with total losses of 13.8%, 19.1% and 24.6%, respectively. In both experiments, the favoured positions for dechlorination were (in order) meta>para>ortho and preference was shown for “open” sites 2 and 3, indicative of the action of 2,3-dioxygenase enzymes [50]. Fava et al. [50] reported that the dechlorination of Fenclor 54 primarily occurred from the meta- and para positions, while ortho-substituted congeners accumulated in the medium. Other studies showed an inability of anaerobic microorganisms to degrade the low chlorinated biphenyls. The occurrence of diortho- and monoorthochlorobiphenyls, as well as the biphenyl rings, was identified even after a one year incubation [31].

### 2.3. Sequential anaerobic-aerobic conditions

Laboratory experiments showed that microbial degradation of lower chlorinated PCDDs/PCDFs and PCBs occurs at a faster rate than in higher chlorinated ones. Lower chlorinated congeners produced by dechlorination can be readily degraded by indigenous bacteria, which in consequence, reduces the potential bioconcentration risk and the exposure to PCDDs/PCDFs and PCBs by conversion to congeners with a low bioaccumulation potential in the food chain [35, 51]. The lightly chlorinated PCDDs/PCDFs and PCBs congeners produced during the anaerobic dechlorination may then be substrates for oxidative destruction by aerobic microorganisms, which leads to the production of chlorobenzoic acid, which is easily degraded by bacteria.

The findings described above indicate that a complete degradation of PCDDs/PCDFs and PCBs can be achieved by sequential exposure to anaerobic and aerobic biodegradation. Highly chlorinated congeners can be transformed to compounds of lower chlorination during reductive dechlorination under anaerobic conditions. Lightly chlorinated congeners, produced during anaerobic dechlorination, might then become substrates for oxidative destruction by aerobic microorganisms, which can lead to the production of chlorobenzoic acid, which is further easily degraded by bacteria [34, 51].

### 3. Physical transformation of PCDDs/PCDFs and PCBs

There is also a division of degradation processes that takes into account the physicochemical degradation of PCDD/PCDF and PCB compounds.

#### 3.1. Photochemical degradation

Photochemical degradation called photolysis also depends on the degree of chlorination, the position of chlorine atoms in the biphenyl ring and the solvent used for PCDD/PCDF and PCB dissolution. The primary process in photoreaction is reductive dechlorination, but examples of photo-induced isomerization and condensation of individual chlorobiphenyls have been also reported.

The first laboratory experiments on photolysis were conducted with mercury lamps as the UV source, with a wavelength of about 254nm, which results in the dechlorination of PCBs. Later, sunlight simulating lamps were used, which also confirmed the degradation of the chlorinated compounds [52-54].

It should also be mentioned that the higher chlorinated biphenyls undergo photolysis faster than less chlorinated ones. For example, the exposure of PCB to a 310nm wavelength causes of reduction of about 70% tetra-, 96% of hexa- and 99% of octachlorobiphenyl. Experiments with tetrachlorobiphenyls showed that the major products after irradiation at 300nm are di- and trichlorinated biphenyls [52]. Bunce et al. [53] reported intensified photodegradation with increased irradiation time.

Photolysis is regarded as one of the major processes reducing PCDDs/PCDFs and PCBs in the environment. Bunce et al. [53] estimated the loss of PCBs in natural waters at the magnitude of 10 to 1000g/Km<sup>2</sup>/year. In shallow water bodies at least one chlorine atom from mostly chlorinated PCB molecules is photodegraded per year. Zepp et al. [54] reported that humic acids and suspended materials may induce and accelerate PCB photodegradation.

Several researchers described accelerated in-situ photolysis by the addition of various organics, such as isooctane, hexane and cyclohexane, on the surface of contaminated soil [56-58]. Dougherty et al. [59] found that solar-induced photolysis reactions can be a principal mechanism for the transformation of PCDD/PCDF to less toxic forms.

### 3.2. Thermal degradation

The last group of PCDD/PCDF and PCB transformations is thermal degradation, leading to the complete destruction of toxic substances at temperatures above 700°C or producing more toxic congeners such as TCDD at temperatures below 700°C. This kind of PCDD/PCDF and PCB destruction is well adapted on an industrial scale for the safe disposal of waste products containing PCDDs/PCDFs and PCBs.

## 4. Environmental biodegradation of PCDD/PCDF and PCB

PCDDs/PCDFs and PCBs are substances that are created during different types of natural and industrial processes. Their appearance in the environment and in consequence in food products creates a serious threat to human health and ecosystem functioning as far as their genotoxic and toxic effects on living organisms are concerned [59]. Therefore, natural transformation of PCDDs/PCDFs and PCBs is a critical event in determining their fate in the environment.

### 4.1. Phytoremediation

Phytoremediation is defined, according to Macek et al. [61], after Cunningham and Betri [62] and Cunningham et al. [63], as the use of green plants to remove, contain, or render harmless environmental contaminants. According to other authors, phytotechnology is a set of technologies that use plants to remediate contaminated sites [64-68].

Phytoremediation uses living plants for the remediation of contaminated mediums, such as soil, sediment, sludge and water (in situ as well as ex situ) by the removal, degradation or stabilization of a given contaminant [64].

According to Macek et al. [61], after Salt et al. [69], phytoremediation is currently divided into several subtypes:

- phytoextraction
- phytodegradation
- rhizofiltration
- phytostabilization
- phytovolatilization

These techniques are an alternative to the widely used methods of physical, physico-chemical and thermal remediation. Their advantages include the possibility of application ex-situ and in-situ, low investment and operating costs with high effectiveness and non-invasiveness in the environment [70-72].

The main problem with the use of phytoremediation techniques is their long operational time and the fact that many of the bioremediation techniques are still in the experimental stage [70-72].

The genesis of the phytoremediation process was observed by the rate of degradation of organic chemicals in the soil with and without vegetation cover. On the basis of the obtained results it was stated that vegetation cover promotes the reduction of organic compounds in soil. Currently, a variety of research indicates the positive effects of using higher plants to degrade organic compounds [73-81].

Siciliano et al. [73] demonstrated the reduction of organochlorine compounds by about 30% during 2 years of plant cultivation; whereas on the soil without plants, the reduction was 2 times lower. Nedunuri et al. [74] reported the reduction of aromatic compounds by about 42% and 50% by using fibre flax (*Lolium annual*) and St. Augustine grass (*Stenotaphrum secundatum*), respectively, over a period of 21 months. Other examples showed remediation of soil contaminated by crude oil using a combination of grass and fertilizers [74-77]. Vervaeke et al. [78] reported a 57% reduction of aromatic compounds and mineral oils during 1.5 years of willow (*Salix viminalis*) cultivation. Pradham et al. [79] demonstrated the usage of phytoremediation as a primary remediation technology and as a final step for treatment of soil contaminated with PAHs. The authors recorded a 57% reduction in PAHs after 6 months of alfalfa (*Medicago sativa*), switch grass (*Panicum virgatum*) and little bluestem grass (*Schizachyrian scoparium*) growth.

A study by Gregor and Fletcher [80] demonstrated the ability of plant cells to metabolize PCBs. While, research by Jou et al. [81] showed the uptake of PCDDs/PCDFs by *Boussonetia papyrifera* growing on highly contaminated soil. The authors reported similar concentrations and distributions of PCDD/PCDF and PCB congeners in plant tissues and soils. Other research demonstrated that several plants of the genus *Cucurbita* (e.g., courgette, pumpkin and squash) can readily take up PCDD and PCDF from soil and translocate them to leaves and fruits [82-84]. It was also found that *Cucurbita* plants can phytoextract PCBs from soil and translocate some quantities to aerial tissues [85, 86]. This confirms that the PCDD/PCDF and PCB contents in plants may closely relate to the surrounding environments where plants grow [81]. Nevertheless, Uegaki et al. [87] reported no concentration differences in brown rice grown in three different soils: dioxin-contaminated soil, paddy soil and upland soil. The authors assumed that growing rice in soil contaminated with high concentrations of dioxins has no influence of the PCDD/PCDF levels in rice tissue [87].

#### 4.2. Rhizoremediation

Rhizoremediation of organic micropollutants is one of the most effective remediation processes due to existing interactions in the rhizosphere between plant roots, plant exudates, soil and microorganisms. Mackova et al. [64] reported that plants support bioremediation by the release of exudates and enzymes that stimulate both microbial and biochemical activity in the surrounding soil and mineralization in the rhizosphere. Plants can also accelerate bioremediation in surface soils by stimulating the growth and metabolism of soil microorganisms through the release of nutrients and the transport of oxygen to their roots [61-62, 67]. Moreover,

the fact that up to 40% of carbohydrates, amino acids and other photosynthesis products are stored in the plant rhizosphere, plays an important role in the availability of carbon used by microorganisms in the co-metabolism process.

A study by Whipps [88] demonstrated that 1g of rhizosphere soil contains a  $10^{12}$  higher amount of microorganisms in comparison to non-planted soil. Microorganisms settling in the rhizosphere also play a role in the protection of plants against pathogens and stress induced by too high a concentration of contaminants and facilitate nutrient uptake by a given plant [89-93].

Bacteria present in the rhizosphere soil serve remediation functions by secreting the appropriate enzymes (e.g., peroxidase, phosphatase, dioxygenase, P450 monooxygenase, dehalogenase, nitrilases and nitroreductase) involved in the degradation of organic pollutants. Such enzymes are also found in plants and fungi that colonize plant roots. This led to a thesis on the interaction of plants and microorganisms in order to completely destroy a given pollutant [93-99]. This process is called rhizodegradation and is defined as the degradation of pollutants in the root zones of plants (rhizosphere).

The effectiveness of rhizosphere biodegradation depends on the ability of microorganisms to adapt to a given pollution concentration and the effectiveness of root colonization [97]. The interactions between plants, soil and rhizosphere microorganisms are multifaceted and according to Macek et al. [61] can give mutual benefit to both organisms. This mutualistic relationship is responsible for the accelerated degradation of soil contaminants in the presence of plants [101]. Research on this issue is ongoing. Already existing publications confirm the validity of the use of rhizoremediation to reduce PCDDs/PCDFs and dl-PCBs. For example, an article by Kuiper et al. [98] demonstrated that naturally occurring rhizosphere biodegradation can be enhanced by the addition of microorganisms to the rhizosphere.

The important group of substances present in the rhizosphere are complexes of aromatic compounds such as flavonoids and coumarins. These compounds are used by bacterial microflora as a source of carbon and nitrogen [73, 98-99, 102-103]. They are structurally similar to organic compounds such as PCBs and PAHs. This indicates the potential of using such evolutionary established metabolic pathways of rhizosphere microorganisms for the remediation of organic pollutants [104]. Thus, many researchers are interested in the ability of microorganisms inhabiting the rhizosphere to degrade organochlorine pollutants and the role of flavonoids and coumarins produced by plants [99, 103, 105-108].

Worldwide studies describe many kinds of pollutants including PCBs, PAH, petroleum hydrocarbons, chlorinated pesticides like Pentachlorophenol and 2,4-Dichlorophenoxyacetic acid, which were more rapidly degraded in the rhizosphere compared to the bulk soil [64, 109-111]. Research by Betts [112] conducted on soil contaminated by petroleum hydrocarbons showed its considerable improvement by using several plants species such as Bermuda grass, rye grass, white clover and tall fescue. A study by Burken and Schnoor [113] described the positive role of root exudates on atrazine uptake by plants (poplar trees). The research also showed that phenolics, flavonoids and terpenes present in root exudates can induce the bacterial degradation of PCBs [61, 114—115]. A study by Mackova et al. [116] showed the effect of tobacco, nightshade, alfalfa and horseradish on PCB removal from contaminated soil. The

obtained results showed 6% to 33.7% removal of PCBs during 6 months of experimentation. The authors also underline the role of the studied plants as a source of bacterial consortia capable of PCB degradation.

## 5. Perspectives in environmental biodegradation of PCDDs/PCDFs and PCBs

PCDDs/PCDFs and PCBs are compounds that occur in all types and structures of ecosystems. Their transfer takes place through biogeochemical cycles, but it is their long half-life in the environment, their accumulation and biomagnification in aquatic and terrestrial food chains and their toxicity that determine their long-term and large-scale threat to the environment and humans. As a result, one of the priority tasks of recent research on PCDDs/PCDFs and PCBs is to characterize the processes that determine their transport and deposition in ecosystems, in order to regulate their allocation and diminish their concentration. Reversing ecosystem degradation and reducing PCDD/PCDF and PCB concentrations in the environment requires solutions based on integrative problem-solving science, such as ecological engineering and ecohydrology [117].

A key element of the ecohydrology theory is the assumption that an excess amount of pollutants including PCDDs/PCDFs and dl-PCBs and their negative effects on the environment can be limited by so-called "dual regulation". Until now, the above methodology was used to reduce the occurrence of toxic cyanobacterial blooms resulting from excessive inflow of phosphorus into water. This concept involves the use of biological and hydrological processes to control the amount and allocation of phosphorus in the ecosystem through increasing biofiltration and by the formation of ecosystem biota [118-120].

Similarly, in order to diminish the concentration of PCDDs/PCDFs and PCBs in the environment there is a need to not only reduce the pollutant load from point and non-point sources but also to develop and apply in-situ bioremediation strategies [72, 117-123]. The application of bioremediation technologies should focus on the possibilities of exploiting and strengthening the functioning of the given ecosystem to reduce the recorded concentrations of PCDDs/PCDFs and PCBs.

The phyto- and rhizoremediation techniques described above are examples of the use of the natural properties of the ecosystem to reduce the environmental PCDD/PCDF and PCB contamination.

Currently, in order to improve the rate and efficiency of such remediation processes a number of advantages have been developed and applied. Some of them are focused on the stimulation of growth and activity in microbial communities in order to accelerate remediation efficiency and diminish the concentration of PCDDs/PCDFs and PCBs in environment.

It should be underlined that there are two main types of microorganism: indigenous and exogenous. Indigenous ones are those that are found already living at a given site. To stimulate the growth of these indigenous microorganisms, the proper soil temperature, oxygen and nutrient content may need to be provided. If the biological activity needed to degrade a

particular contaminant is not present in the soil at the site, microorganisms from other locations, whose effectiveness has been tested, can be added to the contaminated soil. These are called exogenous microorganisms [56]. Research has shown that the stimulation of an indigenous microbial population, by injecting methanol and acetate as an electron donor, enhances the removal of tetrachloroethane (PCE) to ethane [124]. Nevertheless until now, scientists have been faced with the problem of the application of isolated microorganisms *in situ*, as they are often unable to adapt and compete with microorganisms naturally occurring at contaminated sites. This is mainly due to the inability to grow a culture of microorganisms below a certain depth, the lack of sufficient amounts of nitrogen, phosphorus and carbon in the environment, the low bioavailability of pollutants and the preferential use of carbon from non-toxic substrates rather than toxic. An important role is played by the presence of contaminants that inhibit the growth of microorganisms. Currently, in order to avoid such a situation the analogues of the natural soil contaminant are added to the remediated soil. This stimulates the micropollutants' degradation pathways in the microorganisms' cells [99,105,125].

Another problem with bioremediation is the availability of the contaminant to the degrading organisms. To solve this problem research has been conducted on the use of surfactants as potential agents for enhancing solubility and removing contaminants from soil and sediments [126-128]. As reported by Nakajima et al. [129], the addition of sodium dodecyl sulphate, Triton-100 and sodium taurocholate increases the bioavailability of PCBs and PAHs.

Bioaugmentation is another method used in order to improve the microbial degradation of pollutants. This process is based on the introduction of appropriate species for the degradation of specific contaminants. The efficacy of bioaugmentation is contradictory, as far as both positive and negative results have been obtained. A successful bioaugmentation was observed for the remediation of PAHs in sediments [124]. Nevertheless, other studies have achieved no positive results [130].

On the basis of the above data, contemporary bioremediation strategies should be implemented in combination, for example phytoremediation and biostimulation or rhizoremediation and bioaugmentation. This would accelerate the usage of plants and enhance the activity of degrading microorganisms in order to minimize the risk played by PCDDs/PCDFs and PCBs.

It is also possible to remediate soil by using transgenic organisms. Currently, most of the research into the use of transgenic organisms is carried out on a laboratory scale. These experiments are mainly concerned with the introduction of genes encoding biosynthetic pathways of biosurfactants (in order to increase the bioavailability of contaminants), the introduction of genes that enable increased resistance to given contaminants in microbial communities or genes encoding the enzymes' degradative pathways (e.g., cytochrome P450) [131-136].

The latest research by Lan Chun et al. [136] demonstrated the positive role of the electrical stimulation of microbial PCB degradation. The authors found a 40-60% reduction in total PCB concentration in weathered sediments exposed to electric currents, while no significant decrease in PCB concentration was observed in control sediments.

The techniques described above and their advantages, such as biostimulation and bioaugmentation, can be adopted and used in large-scale remediation processes. Examples of such an approach include the utilization of wetlands and biofilters.

Wetlands are often described as "the kidneys of the landscape" owing to their the intrinsic function to transform and store organic matter and nutrients [138] and associated micropollutants such as PCDDs/PCDFs and PCBs. This ability has been exploited for water quality improvement [138]. Constructed wetlands were first used for wastewater treatment in the 1950s. In recent years constructed wetlands have been widely used for urban and agricultural runoff treatment. They utilize natural processes to purify water in a sustainable, cost and energy effective way with minimal operation and maintenance cost [140]. Furthermore, the usage of constructed wetlands as tools in the treatment of polluted waters, has been gaining popularity as an ecological engineering alternative over conventional, chemical based methods [141-142]. Several scholars have shown successful utilizations of constructed wetlands for the treatment of a wide variety of wastewaters including industrial effluents [142-144], urban storm water, agricultural runoff [146-147], domestic wastewater [148] and animal wastewaters [149]. Schulz and Peall [150] determined the effectiveness of constructed wetlands in retaining agricultural pesticide pollution as 89% during runoff. Several researchers have proven the ability of constructed wetlands to mitigate pesticide pollution derived from various agricultural nonpoint sources [151-155]. Considering the above, it appears that the use of constructed wetlands to purify water from organochlorine compounds is a promising challenge.

Furthermore, the use of land-water ecotones constructed in a river valley with different kinds of plants and microorganisms may partially purify the inflowing surface- and groundwater contamination by PCDDs/PCDFs and PCBs [156-157]. Such structures may capture, immobilize and/or degrade PCDDs/PCDFs and dl-PCBs [61, 96, 103].

The other promising solution involves the use of biofilters for the purification of inflowing water, wastewater, leachate etc. Such biofilters combined with areas of intensive sedimentation, which enable the deposition of matter, nutrients and micropollutants and their further biodegradation by existing microbial consortia and areas of macrophyte growth, wherein intensive phytodegradation processes occur, are considered to be one of the most effective solutions for pollutant removal. Results obtained by Urbaniak et al. [158] in the Asella Demonstration Project demonstrated changes in the Toxic Equivalent (TEQ) of PCDDs/PCDFs in the sediments of the Asella river and lake taken before and after biofilter construction. Authors showed a 70% reduction in sediment toxicity after one year of biofilter implementation. This indicates the positive role of biofiltration in the quality of lake ecosystems and in consequence on human health. The implementation of such biofiltration system enabled a reduction in the input of PCDDs/PCDFs into the lake through sedimentation and due to acceleration of photo- and biodegradation processes the quality of the whole river-lake system was improved.

## 6. Conclusions

PCDDs/PCDFs and PCBs pose one of the most challenging problems in environmental science and technology. Their fate, transport and biodegradation in the environment occur via complex networks, involving complicated interactions with other contaminants and with

various physiological, chemical and biological processes. Those processes can be used and modified in order to diminish their environmental concentration. The promising results of such activities performed by researchers worldwide were described in this chapter. Nevertheless, the still existing challenge is to develop a bioremediation strategy that involves and integrates different types of solutions, on the scale of the whole ecosystem, in order to optimize the effectiveness of pollutant removal.

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