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Effect of PCP Pesticide Contamination on Soil Quality

Rim Werheni Ammeri, Yassine Hidri and Hassen Abdenaceur

Abstract

In recent years, soil contamination with pesticides has become a crucial news issue with serious short- and long-term effects on human health and its environment. Pesticides play a significant role in the success of modern farming and food production. These compounds have potential for toxicity and adverse effects on human health and ecological soil systems. Pentachlorophenol (PCP) is one of the most recalcitrant chemicals polluting the environment for its stable aromatic ring system and chloride content. Nowadays, many sites are contaminated with this substance. In these areas, concentrations may stay high for a long time because of slow degradation in the soil due to the negative effects that PCP has on soil microbial populations. Bioremediation of PCP contaminated sites can be realized introducing directly, into a contaminated system, microorganisms able to consume selectively the target compound (bioaugmentation) or increasing the microbial indigenous population by addition of nutrients in form of organic and/or inorganic fertilizers and biosolids (biostimulation). In the present chapter, we present an overview of the effect of PCP pesticide contamination on soil microbial populations (density and diversity), enzymatic activity and physicochemical parameters. Additionally, the bioremediation process will be detailed.

Keywords: soil contamination, pentachlorophenol, bioremediation, microorganisms, ecological system

1. Introduction

Soil is an active, dynamic, and nonreplaceable reserve, and its situations impact its construction, environmental efficacy, and total stability [1, 2]. The charge of soil reposes on in portion on its ordinary structure and on the variations affected by human use and administration [3]. Soil, as the main interface with other environmental compartments, plays an important role in the fate of organic pollutants. During the treatment of crops, most of the quantities of pesticides applied reach the soil, either because pesticides are directly applied to it, or because the rain has washed the foliage of treated plants (crops and/or weeds). The ground therefore occupies a position central in regulating the fate of pesticides in the environment, and it will have a dual role of storage and purification [4].

Pollutants come into contact with soil primarily through deliberate application, dispersion, and atmospheric deposition. The soil therefore represents a storage reservoir for these substances. These compounds can also be lost from the soil or remain at high concentrations. Consequently, the fate and behavior of organic contaminants in soils have been the subject of intense research, with particular

interest in the bioavailability of these contaminants [5]. The greatest difficulties in studying and estimating the retention and degradation of pollutants in soil are the diversity of chemical structures and reactivity of these compounds on the one hand and the high level of diversity of soils in terms of structure and composition, soil, and climatic conditions, especially soil temperature and humidity on the other [6]. Soil pollutants are very diverse, and they are also often harmful and toxic to all living forms and more specifically humans (Roger and Jacq, 2000). The occurrence of pollution most often results from industrial accidents, deposits, or the transport of dangerous materials [7]. Like pesticides, chlorinated solvents, nitrogen, and certain trace elements such as copper, mercury, or silver, pollutants can both be naturally present in the soil or be the result of human activities. This generalized use of PCP has led to the contamination of water and soil systems, with PCP currently considered to be a product of priority for decontamination studies [8]. These compounds are, in fact, the source of many concerns for companies operating in the wood preservation sector. Chlorine compounds are harmful, and they are found in the effluents of many industries, such as the chemical and petrochemical industries, those of resins and coking plants, pesticides, textiles, paper, and even in the pharmaceutical industry and many others [9]. This is why these chlorinated compounds appear as the most frequently encountered pollutants in various natural environments such as forests, rivers, marine waters, industrial discharges, urban effluents and even in the groundwater. The soil has a marked self-purification capacity. It is in fact capable of degrading the polluting compounds or immobilizing them inside it so that the volatilization and leaching processes are drastically reduced to the benefit, also, of the other environmental sectors. However, an excess of pollutant exceeded the soil storage capacity or a change in environmental conditions [10]. Soil, as the main interface with other environmental compartments, plays an important role in the fate of organic pollutants. Pollutants come into contact with soil primarily through deliberate application, dispersion, and atmospheric deposition. The soil therefore represents a storage reservoir for these substances. These compounds can also be lost from the soil or remain at high concentrations. Consequently, the fate and behavior of organic contaminants in soils have been the subject of intense research, with particular interest in the bioavailability of these contaminants [5]. The greatest difficulties in studying and estimating the retention and degradation of pollutants in soil are the diversity of chemical structures and reactivity of these compounds on the one hand and the high level of diversity of soils in terms of structure and composition, soil, and climatic conditions, especially soil temperature and humidity, on the other [6].

2. Soil and pesticide pollution

Vigorous soil is a necessity for a healthy existence. Vigor, value, and sustainability of soils are contingent on their physical, chemical, and biological variety. Hence, soil biodiversity that really tops midair biodiversity is vital for ecosystem permanence and service area. A detailed association occurs among soil biodiversity and agricultural soil organization [11]. Pesticides or biocides are chemicals, organic or inorganic, intended for the fight against undesirable organisms such as bacteria, fungi, insects, and weeds. The use of pesticides appears beneficial, or in the absence of treatments, the yields of agricultural production and quality (essentially the development of crops in the agricultural sector) or industrial (such as wood treatments and railways, textiles and food), would be reduced and/or weakened. Over 500 different pesticide formulations are authorized worldwide to control different types of pests in the agricultural sector [12]. Pesticides are classified into different

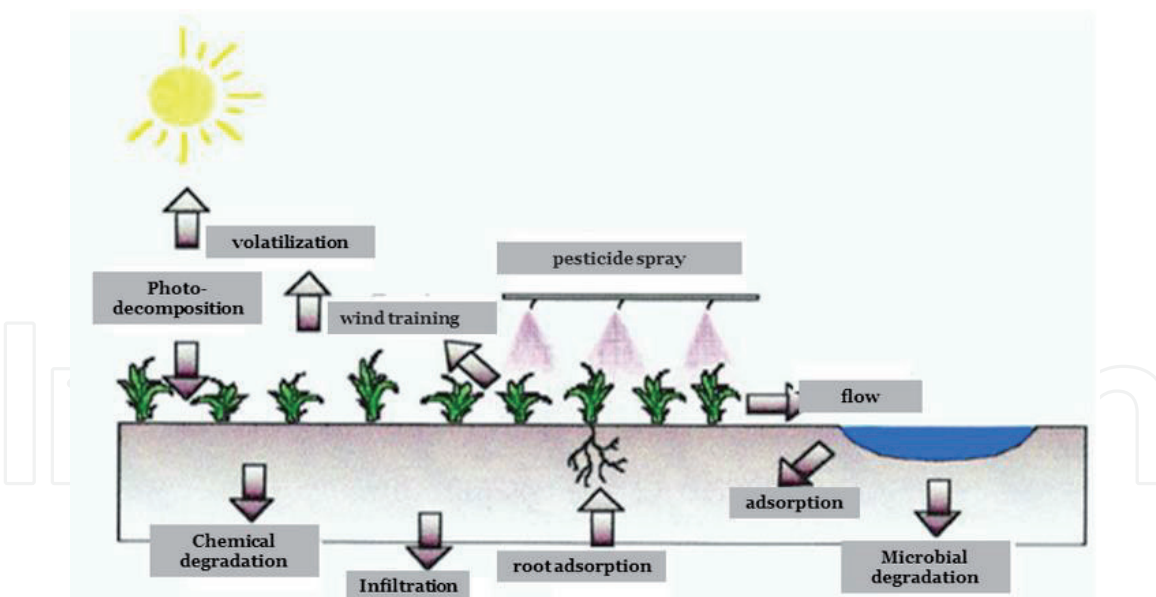


Figure 1.
 Behavior of pesticide molecules in the natural environment [17].

categories according to their target, their mode of action, their time of action or their chemical nature, and recently, in response to pressure social stressing the danger of pesticides mainly for humans and the environment [12]. In this study, we will focus more specifically on the study of a pesticide very well used in the wood treatment industries, namely PCP. Soil pollution is often thought because of chemical contamination. The use of poor-quality water and application of excessive amounts of pesticides and fertilizers can result in soil contamination. To some degree, most of the soils are capable of adsorbing and detoxifying many pollutants to harmless levels through chemical and biochemical processes. Polluted water and soil pose a serious threat to plants, affecting the yield [13]. Furthermore, soil has the ability to adsorb pesticides in the humus and clay contents [14]. However, soil plays an important role in pesticide degradation [15].

The dispersion of pesticides in the various compartments of the ecosystem (air, soil, and water) is very rapid [4, 16]. When an organic compound is applied and penetrates into the soil, in substance, it may be subject to relocation or alteration of its chemical structure. These mechanisms can be on the one hand abiotic, of a physical nature (volatilization, adsorption by the soil, leaching, etc.) or of a chemical nature (hydrolysis, photodegradation, etc.) and on the other hand biotic when it occurs. Acts of absorption and metabolism by the various microorganisms living in the medium (**Figure 1**). All of these processes are strictly influenced by the physicochemical properties of the soil and pesticides as well as environmental factors [18].

3. Pentachlorophenol and soil contamination

In 1936, the American company Dow and Monsanto Chemical introduced PCP [19]. Due to its high availability and very favorable price, it has been increasingly used in different functions in several countries around the world. Its main use was accentuated in the wood industry as a preservative (80%). PCP (C_6Cl_5OH) is a highly substituted aromatic compound, prepared by reacting chlorine with phenol in the presence of a catalyst at high temperature and does not have isomers. It was last manufactured in Canada in 1983. Petroleum oils used as a carrier for PCP are generally sourced from

Canadian sources. PCP is solid at room temperature. It is a stable organic compound, slightly soluble in water and highly soluble in organic solvents. Also, PCP a proven carcinogen, immunotoxic, produces an oxidative stress and metabolic disorders [20]. PCP is a highly recalcitrant compound with toxic and carcinogenic properties. PCP is a respiratory poison with both noncarcinogenic and carcinogenic health effects. The PCP molecule can be an endocrine disruptor and inflict high toxicity on all types of organisms [21].

Due to its toxicity and carcinogenicity, as well as the large number of known sites contaminated with PCP around the world, it has been placed on the Priority Pollutants Worldwide List. The products treated with PCP are mainly telephone and power line poles and railway ties. It has therefore become the preferred impregnation product for a wide variety of other special purpose products, such as guardrail posts, signposts, retaining walls [22]. It is also used as an antimicrobial agent in industrial cooling systems, in food packaging, as the main active ingredient in exterior stains and paints. It is found in dental care products [23], in antibacterial soaps, in dermatological medical products [24] and as agricultural biocides and fungicides. PCP is toxic to humans as well as to animals. PCP toxicity is due to the fact that it decouples oxidative phosphorylation making the cell membranes permeable to protons and thus dissipating the gradient transmembrane of H^+ ions and electric potential [25]. It is therefore responsible for alterations in the functionality of the membranes [26]. PCP can be absorbed by mammals through the skin from the ground; it is corrosive to the skin and can cause burns and blisters. In mammals, acute exposure can increase body temperature, causes breathing difficulties, increase blood pressure, causing hypoglycemia and cardiovascular stress [24]. Chronic exposures to PCP can have serious adverse health effects. PCP is a carcinogenic, teratogenic suspect and is highly embryotoxic in addition, potential chronic effects can include kidney, liver, lung and system damage central nervous [24].

Environmental pollution from PCP can occur due to release into the environment during the production, storage, transport or use as a preservative of the wood in place. Also, the production of its sodium salt and the secondary use as fungicide, bactericide, algicide, herbicide, etc. can cause environmental pollution.

The PCP then enters the surface and deep waters of factories, wood treatment plants, and sites for the accumulation of hazardous waste or for spillage, disposal of hazardous waste and for its use as a pesticide. In soils, due to the stability of its structure and high degree of chlorination, PCP is persistent in the environment and is one of the most common soil contaminants. The dispersion of pesticides in the various compartments of the ecosystem (air, soil, water) is very rapid [16]. When an organic compound is applied and penetrates the soil, in substance, it may be subject to relocation or to a change in its chemical structure. These mechanisms can be on the one hand abiotic, physical in nature (volatilization, adsorption by soil, leaching, etc.) or chemical in nature (hydrolysis, photodegradation, etc.), and on the other hand biotic when it acts of absorption and metabolism by the various microorganisms living in the environment. All these processes are strictly influenced by the physicochemical properties of the soil and pesticides as well as environmental factors [18].

4. Pesticide PCP effect in bacterial ecological system

Microbial communities in soils are among the most diverse on Earth [27]. In doing so, soil microorganisms mainly perform several soil functions such

as the nutrient cycle and the detoxification of terrestrial ecosystems [28]. By affecting this diversity, contamination of natural environments constitutes a significant risk that can reduce the ability of ecosystems to resist and recover from the various disturbances they must undergo. The diversity of natural ecosystems is therefore an asset to be preserved. Indeed, it has been shown that the most diverse ecosystems are the most resistant and resilient to natural and anthropogenic disturbances. Since the start of the industrial era, the diversity of natural ecosystems has been in constant decline due to, among other things, contamination of soil, air, and waterways. In order to predict the effect of a substance on a biological community and thus control or limit its use, it is necessary to produce toxicological information on a wide range of organisms. Over 1.75 million different species have been listed for the eukaryotic domain alone [29]. The total number of eukaryotic species has been estimated by several authors and is generally between 5 and 10 million [30]. With regard to prokaryotes (archaea and bacteria), 10,000 species have been described, but this could constitute only around 0.1% of the total diversity of these two domains [31], for an approximate total of around 10 million. It is important to mention that the concept of species in biology and microbiology is different [32]. Each gram of soil can contain more than 1000 species of single-celled fungi [33] and 6000 species of bacteria [34]. This genetic (and therefore metabolic) diversity allows microbial communities to be involved in a multitude of processes that allow ecosystems to function well. Soil microorganisms are important contributors to the different biogeochemical cycles of carbon, nitrogen, and phosphorus in soils [28]. It has been estimated that this community could withstand between 80 to 90% of the biochemical reactions occurring in the soil [35]. Communities of soil microorganisms, via their diverse metabolic capacities, also show a response to soil pollution and thus participate in the detoxification of natural environments [35].

The communities established in polluted soils are very different from those present in unpolluted soils, whether from the point of view of total abundance or specific diversity [36]. It follows from these disturbances of communities of microorganisms a modification of the enzymatic activities carried out by the microorganisms [37]. The specific diversity and the total abundance of microorganisms can be influenced by pollution. This reaction would depend both on the nature of the pollutants and their abundance [37].

In the environment, PCP is a topic to a diversity of biological and physico-chemical procedures, counting biodegradation, photodegradation, evaporation, and sorption, and leaching [38]. These procedures happen in all kinds of natural ecosystems with variable efficacy and have a direct influence on the last rate of this chemical. The main way to eliminate PCP from the environment is through biodegradation by microorganisms [39]. Studies with experimental ecosystems have designated that ecological properties may occur at PCP levels as low as those causing chronic toxicity in sensitive species in single-species tests [40, 41]. The final rate that produced adverse effects in these studies was $15.8 \mu\text{g/L}^{-1}$, which caused a reduction in numbers of individuals and species in a marine benthic community [42]. The diversity and activity of microorganisms in the soil effect the working of ecosystems and thus plant development and health, including the quality and quantity of the crop yield [43]. However, the variety and movement of microbes are actually prone to various stresses counting chemical pollution [44]. The attendance of soil bacteria can improve the extent of pesticide degradation [15] as well as degradation of other organic pollutants [45].

5. PCP degradation

In the soil, pesticides are affected by diverse physical, chemical, and biological procedures, which will condition their degradation, their transmission to other compartments of the environment (water, plant, and atmosphere) and thus their potential influence on exposed living beings [4]. The behavior of pesticides will be more particularly controlled by the phenomena of retention on soil constituents (organic matter, clays) and degradation [46].

The remediation of a PCP contaminated site can take place through abiotic processes such as volatilization, photodecomposition, and immobilization in the soil. There biotic degradation can occur through absorption by plants or animals and through microbial degradation. Three processes are responsible for PCP biodegradation: hydroxylation, oxygenation and dechlorination. The most common formulation for PCP is that of sodium salt which, being not volatile, causes that the contribution of volatilization to the entire abiotic degradation is normally negligible [24]. The biological degradation of pollutants in the soil, or biodegradation, is carried out by living organisms and / or by the associated enzyme kit. During the biodegradation process one or more organisms metabolize the contaminant in an inorganic compound (such as CO_2 , H_2O , NH_3), the autotrophs derive the necessary resources for their growth and development [47]. This catabolic activity of which microorganisms are capable, and which allows them to degrade the contaminants present in the soil, is fundamental for the fertility and health of soils. Several researchers have developed methods to treat and degrade PCP, among these techniques the use of Fenton reagent [48], photocatalytic degradation using TiO_2 [49], the combination of the two methods, namely the Fenton reaction and photocatalytic degradation [50], the ultrasonic method recommended by Francony and Pétrier [51] and also by ozonation. Another method, using the purifying capacities naturally present in certain organisms, is bioremediation. In this case, it is the microorganisms present in contaminated environments that are used to degrade the pollutants (**Figure 2**). Bacteria play an important part in this natural decontamination, due to their ability to evolve very quickly in the presence of selection pressure. Indeed, thanks to point mutations, endogenous rearrangements and horizontal transfers, they can adapt to the presence of pollutants by developing the enzymes making it possible to degrade and/or use this pollutant for their survival and their

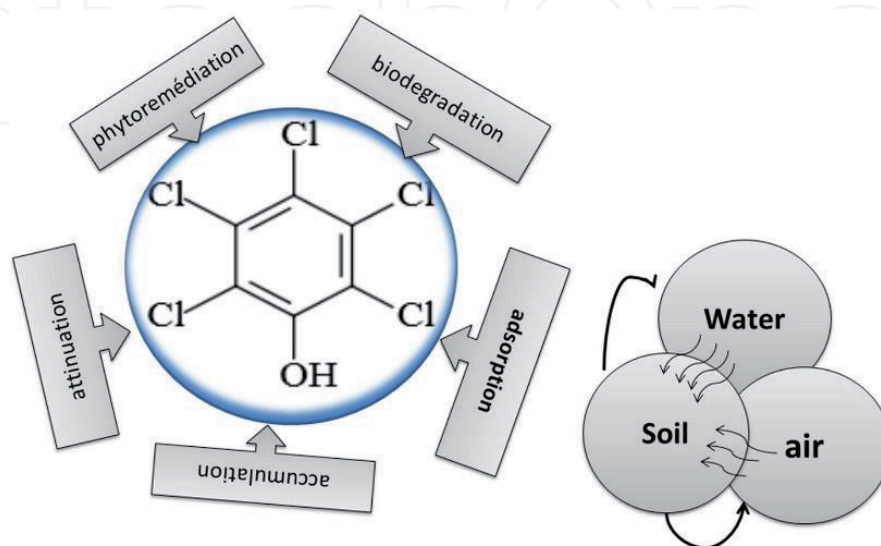


Figure 2.
PCP interactions in environment.

development. Thus, sometime after the appearance of xenobiotic molecules having no equivalent in nature, we can witness the appearance of new metabolic pathways allowing the degradation of this compound [52]. Thus, microorganisms can adapt to resistance to a broad spectrum of diverse pollutants [21]. They therefore constitute an interesting path in the development of natural techniques for removing pollutants.

Bioremediation can be carried out in different forms: natural attenuation, biostimulation and bioaugmentation. Natural attenuation is a process that uses the capacities of microorganisms present in polluted ecosystems or soils. Even if this decontamination technique does not theoretically require human intervention, it is nevertheless necessary to eliminate or neutralize the source of pollution and to constantly monitor the site until the end of treatment [53].

This type of bioremediation is very inexpensive, since it does not require a lot of resources, but it does require long periods of treatment. Biostimulation is the stimulation of the native microflora by adding nutritive molecules, specific or not, to promote bio-pollution (ex-situ or in-situ). Bioaugmentation consists of the addition or inoculation of specific bacterial cultures to stimulate the biodegradation used in bioreactors and ex situ systems.

Numerous works have monitored PCP removal by bacteria and fungi and the usage of plants for its biological elimination [54–57]. Organic objects such as wood chips, sawdust, straw of wheat have been revealed to motivate microorganisms in the removal of PCP in soil [58, 59].

6. Some enzymes responsible for the degradation of PCP

For the bacterium *Sphingomonas chlorophenolica*, the first step is catalyzed by the enzyme PCP-4 monooxygenase encoded by the *pcpB* gene. According to Orser et al. [60], the *pcpB* gene would probably be present on the chromosome and not on a mobile element (plasmid or operon). The degradation of PCP by *Sphingomonas chlorophenolica* sp. nov is carried out via four structural genes *pcpA*, *pcpB*, *pcpC*, and *pcpD* as well as by the regulatory gene *pcpR* [61]. Transcription of the *pcpB* gene is induced by the presence of PCP in the bacteria *Flavobacterium* sp. ATCC 39723 [60]. The presence of PCP is also necessary in the bacterium *Rhodococcus chlorophenolicus*, during the degradation of several chlorophenols [62]. For the *pcpA* gene, its location relative to that of *pcpB* is not yet known. This enzyme is responsible for the conversion of di-p-Hydroquinone to chloromaleylacetate. The *pcpB* enzyme is responsible for the conversion of PCP to tetrahydroquinone via the elimination of chloride ions and by hydroxylation at the para position [63]. Subsequently the enzyme tetrachloro dehalogenase reductive (*pcpC*) converts tetrachlorohydroquinone into trichloro-chlorohydroquinone and itself responsible for the conversion into trichlorohydroquinone (TeCHQ) into dichloro-p-hydroquinone (2,6-DCHQ) or the compound TeCHQ is sequentially dehalogenated. The latter compound is converted into chloromaleylacetate by the enzyme 1,2-dioxygenase (*pcpA*) which will subsequently be converted into 2-maleylacetate by the enzyme *pcpE*; this degradation of 2,6-DCHQ occurs by cleavage of the cycle, leading to the formation of 2-chloromaleylacetate which is more degraded via the Krebs cycle [63]. The presence of an electron donor and acceptor is essential. Biodegradation would not occur if one of the two is missing. The degradation rates of organic compounds depend on their chemical structure. The more a molecule is substituted, the more difficult it is to degrade. The position of substituent also plays a role since the ortho and meta positions increase the resistance of the molecules, as well as the

substitutions on alpha carbon, compared to that in omega. Under aerobic conditions, substituted chlorine inhibits the activity of monooxygenase and dioxygenase, which are the main aerobic cleavage enzymes of the benzene nucleus. PCP is resistant to aerobic degradation. Reductive dechlorination has been suggested to be the first step in the biodegradation mechanism of PCP [64]. Low-substituted chlorophenols are more sensitive and labile to aerobic degradation [65]. The optimal condition is that these reductive dechlorination metabolites are subsequently degraded via the aerobic process. This can reduce the inhibition produced by the dechlorinated intermediates. The progressive mineralization of the component generates a series of microbial communities and enzymatic activities that enables an efficient dissipation of pesticides in soil and avoids metabolite accumulation [45].

6.1 PCP effect in enzymatic soil activity

It must also take into reason that soil is a specific active micro-habitat, everywhere organic and inorganic constituents, microbes, enzymes, nutrients, and environmental influences collaborate with each other and alteration with period and place. Evidently, these communications can control spatial variety of soil bacterial communities and enzyme activities and affect their appearance and association levels, in turn depending on diverse soil properties [66]. Consequently, difficulties in the approximation of the total bacteriological community and its dynamic portion, characterized by enzyme actions, can raise level if progressive methods have been utilized in their control. Many studies recommended that soil enzyme activities as appropriate and reliable indicators of soil quality by Gianfreda and Bollag [67] and Drijber et al. [68]. The study of Siczek et al. [40, 41] improved that the soil biological parameters can increase the activities of the enzymes involved in the N and P cycle (protease and acid phosphomonoesterase) and total activity (dehydrogenase). Some biological analysis confirmed that the addition of PCP had a significant impact on the metabolic potential of soil bacteria. Several studies have described changes in the enzymatic activities of soil contaminated with PCP [69]. PCP degradation is a process that can be completed through three ways: oxygenolysis, hydroxylation, or reductive dehalogenation [70] (**Figure 3**). Since, soil microorganisms can produce various extracellular compounds like oxidoreductases, such as peroxidases, laccases, and tyrosinases. The laccase is known as the benzene-oxygen oxidoreductase; EC 1.0.3.2. has been subjected to intensive research in the last decades. This enzyme oxidizes a great variety of aromatic compounds with a concomitant reduction of oxygen to water [71, 72]. Thus, this kind of enzyme is involved in the oxidative coupling processes of chlorophenols [73]. The residual products of enzymatic reactions, laccase, and peroxidase are usually less toxic than the parent components according to Gianfreda and Bollag [74]. PCP removal from soil can occur either by abiotic [58] or enzymatic oxidative processes [75]. According to Liang et al. [76], the incorporation of some organic compounds to soil allowed effectively stimulation of the dehydrogenase activity since the added organic material may contain some intra- and extracellular enzymes allowing stimulation of the microbial activity in the soil. Also, bioaugmentation is known as a bioremediation choice allowed by increasing the natural in-situ microbial population in the polluted environment [77].

PCP also troubled the activities of intracellular enzymes, which are measured to be an indicator of the active microbial biomass, since they are active within the living cells of microorganisms [40]. Zhang et al. [78] originate that phenol contaminants (including PCP) significantly reduced dehydrogenase, respiration, and urease activity in comparison with soil, which had not been contaminated. As dehydrogenase contributes to the biological oxidation of soil organic matter by hydrogen

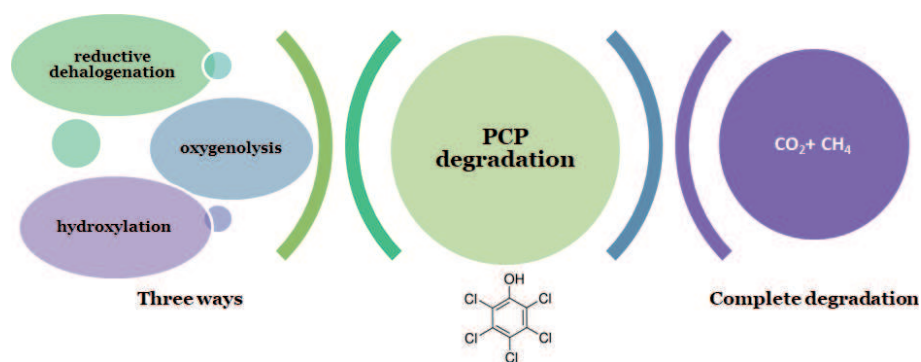


Figure 3.
 PCP degradation ways.

relocation from the organic substrate to inorganic acceptors, the lower activity of this enzyme could designate an inferior rate of decomposition of soil organic matter after PCP treatment. A similar conclusion may be drawn from a respiration analysis; this activity was also reduced by PCP [40, 41].

On the other hand, PCP increased the amount of phosphorus transformation, as showed by an acid phosphomonoesterase analysis showing an important coefficient correlation ($r = 0.850$) with PCP. Wang et al. [79] showed the opposite effect of PCP on acid phosphomonoesterase was create in our study (it increases activity), which could be the result of different soil properties and different experimental conditions.

In a micro-environment study, the destructive effect of PCP on manganese peroxidase activity was controlled during the first 14 days, though, after that period the movement augmented [58]. Additionally, laccase movement decrease to PCP. A laboratory research presented that the influence of PCP on enzymes was reliant on its rate [80]. A study by Urrutia et al. [57] achieved with rhizotrons showed that here was no impact of growing PCP rate in soil from 50 to 250 mg kg⁻¹ on the microbial biomass in the ryegrass rhizosphere. However, PCP negatively affected soil activity through reducing the dehydrogenase as well as β -glucosidase activities as the PCP rate augmented. A considerable rate of literature has been published in relation to the influence of organic contaminants including PCP on soil enzyme and microorganism activity [41, 81]. However, some studies mainly concerned laboratory experiments, proved that PCP significantly reduced dehydrogenase, respiration, protease, urease, and β -glucosidase activity. This shows that PCP was a substantial factor in decreasing microbe activity in soils [80].

6.2 PCP effect in physicochemical parameters

PCP absorbs to organic matter causing removal of PCP from water into sediment depending on the chemical structure and environmental conditions[82, 83]. Bio-elimination of chemicals arises through the actions of logically arising microorganisms and biomass population. Soil influences, such as moisture content, pH, and temperature, also show a significant character. The removal is improved in the soil pH range of 5.5–8.0, with an optimal value of about 7 [84], and tends to rise with temperature [85]. The result of soil moisture satisfied on the biodegradation of pesticides, though, is not completely assumed. It is acknowledged that the accessibility of soil moisture is obligatory for improved biomass movement. The amount of pesticide removal under saturated soil situations is also acknowledged to be very slow [86]. With upper soil moisture content and soil temperature in the summer months, the pesticide may destroy quickly, thus dropping the hazard of water pollution. It can, though, be renowned that the moisture content in the

soil profile is not preserved at the similar level during sub-irrigation; it is close to saturation near the water table and reductions with distance overhead the water table. Yet, when the soils had a low level of organic matter (>10%) will be take a great affinity for organic pollutants due to the presence of humic acid, fulvic acid, and reactive clay such as Al and Fe hydroxide groups [58]. In the literatures, there are plenty of studies indicated that denitrification can be disturbed by several environmental pollutants, such as heavy metal and synthetic organic compounds [87]. For example, the Zinc oxide nanoparticles have been observed to inhibit the denitrifying reductase, which further led to more nitrate accumulation. Zheng et al. [88] found that, it is essential to explore the effect of PCP on the metabolism and function of denitrifying bacteria. The contact of PCP to *P. denitrificans* bacteria induced the reduced the key enzymes activities connected to glycolysis process, caused the trouble of the metabolism of glucose utilization and the cell growth, and subsequently disturbed the generation of electron donor (NADH) for denitrification via NAD⁺ decrease. Denitrification procedure was significantly inhibited by the PCP at upper amount of PCP, which would further disturb the nitrogen cycle in soil [89].

This may indicate that less nitrogen was available for the plants, and that the plants contaminated with PCP may suffer from nitrogen deficiency, which confirmed our analysis of plant N content. It is worth noting that relatively speaking the most harmful effect of PCP was noted for enzymes related to the nitrogen cycle, e.g. protease and urease [57]. In satisfactory situations of development e.g. pH, temperature and moisture and adequate supply of nutrients like vitamins, magnesium, manganese, copper, sulfur, potassium, phosphorus and nitrogen, microbes can biodegrade/biotransform the complex hazardous organic chemicals into simpler and harmful ones. After the usage of “super bug” in elimination of oil spills, there has been numerous efficacious stories of microbial method in clean-up of polluted lands and soils [90]. The Microbiological Resource Centers (MIRCENS) at Cairo, Egypt is examining the use of microbes in degrading persistent pesticides pollutants (UNEP Reports, 1996–2006).

6.3 PCP bioremediation in soil

Soils are open, porous, multi-compound of biogeochemical systems containing solids, liquids, and gases [91]. At the same time, they are a preferred sink for dangerous pollutants like hydrophobic organic compounds and multiple other compounds that are increasingly finding their place in the environment [92, 93]. The main difficulties encountered in biological treatment methods are the lack of knowledge concerning the bacterial population degrading PCP under unfavorable environmental conditions [94, 95]. Biodegradation is a biological degradation carried out by living beings (bacteria, fungi, plants, etc.). It is due to the abundance and variety of organisms in the environment considered [96]. For example, the attack of a chemical molecule by microorganisms often results in its mineralization and the production of low molecular weight metabolites (**Table 1**). Two types of biodegradation are most often cited and distinguished:

Primary biodegradation: It corresponds to metabolism and co-metabolism. These can be done by substitution or rearrangement of the structure of the compound, by redox or isomerization, or by addition and loss of substituent. This is a partial attack on the molecule. In some cases, it can lead to the appearance of persistent metabolites, more bioavailable and/or more toxic than the initial molecule.

Ultimate biodegradation: It is a complete degradation leading to the formation of carbon dioxide, methane, water, and mineral elements. This biodegradation, if it

Bacterial strains	Degraded chlorophenols	References
<i>Pseudomonas</i> sp. UG25 et UG30	PCP	[97]
<i>Pseudomonas</i> sp. RA2	PCP	[98]
<i>Pseudomonas</i> sp. strain SR3	PCP	[99]
<i>Pseudomonas</i> sp. strain IST103 PCP	PCP	[100]
<i>Pseudomonas mendocina</i> NSYSU	PCP	[101]
<i>Mycobacterium</i> sp. strain CG-2	PCP	[102]
<i>Mycobacterium chlorophenolicum</i> PCP-1	PCP	[103]
<i>Sphingomonas</i> sp. strain P5	PCP	[104]
<i>Sphingomonas chlorophenolica</i> RA2	PCP	Nohynek et al., 1995; Ederer et al., 1997; [103]
<i>Novosphingobium lentum</i> MT1	PCP	[105]
<i>Sphingomonas chlorophenolica</i>	PCP	[106]
<i>Rhodococcus</i> sp. CP-2 et CG1	PCP	[102, 62, 107]
Strain KC-3	PCP	[108]
<i>Flavobacterium</i> sp. ATCC39723	PCP	[109, 60]
<i>Flavobacterium</i> sp.	PCP	Gonzalez and Hu, 1991
<i>Flavobacterium</i> strains	PCP	[110, 111]
<i>Arthrobacter</i> strain NC	PCP	[112]
<i>Corynebactrium</i>	PCP	[108]
<i>Burkholderia</i>	PCP and CP	[113]

Table 1.
Examples of some bacterial strains competent to degrade PCP.

occurs quickly, leads to the total elimination of the pollutant from the environment. A substance that undergoes ultimate biodegradation is one that poses less risk to the environment than a substance that undergoes primary biodegradation.

Bacteria can feed on all kinds of compounds. These are what we call electron donors. In addition, they can breathe with different compounds. These are the electron acceptors. In the case of stimulated biodegradation, the electron donor or electron acceptor is contamination. In this context, several researchers have focused their attention on studying microbial biodegradation which has been reported as a main mechanism of the dissipation of pesticides in the soil environment ([114]; Pieuchot al., 1996). As an electron acceptor or as an electron donor, the degradation of these molecules is an integral part of metabolism and directly serves the production of energy for microorganisms. The substance appears to be metabolized by the body. A compound is said to be biodegradable if it is completely transformed by living organisms into CO₂, H₂O, and cellular biomass. Mineralization corresponds to the bioconversion of organic matter into mineral products (CO₂, CH₄, H₂O, NH₃, HCl, etc.). It is the reverse biological process of the synthesis of organic matter (mainly photosynthesis and methanogenesis). Some molecules are resistant to any degradation action over very long periods. The stability of these molecules is linked to their chemical structure, their concentration, and the characteristics of the surrounding environment. Generally, the more a molecule is substituted, the

more it is resistant to biodegradation. The position of the substituents also plays a role [64]. Replacing carbon with other atoms such as O, N, S, such as multiple branching on the same carbon atom, changes the resistance to biotransformation of organic products. The presence of the substrate in too high concentration may result in the inhibition or inactivation of one or more enzymes involved in microbial metabolism. Many species of soil bacteria have been isolated from samples of soil contaminated with PCP (**Table 1**).

Whereas microbial remediation (bioremediation) is a fixed technology for the removal of organic soil contaminants, the use of microorganisms to transform organic contaminants similar PCP is still being explored. Bioremediation of soils includes numerous technologies, counting bioaugmentation and also biostimulation, to augment the elimination of PAHs. Bio-augmentation, it is the addition of microorganisms that biodegrade (toxic organic compounds) a specific contaminant. Microbial remediation depends upon the appearance of suitable microorganisms in the correct amounts and in mixtures and in appropriate environmental conditions. Biostimulation and bio-augmentation are two indispensable factors inducing bioremediation by microbes. In the bio-Stimulation procedure, the adding of the amendments serves to rise the number or activity or both, of naturally happening micro-organisms available for bioremediation. The in-situ bacteriological remediation approaches might necessity to combination with phytoremediation process with suitable hyper-fixator plants that can successfully acceptance the pollutant (made bioavailable by the microorganisms) from soil and bioaccumulate them in their roots and shoots, thus stopping their reprocessing in soil. Bioremediation is the procedure by which active organisms destroy or transform hazardous organic contaminants to inorganic components, such as CO_2 , H_2O , and NO_3^- [115], which are also formed during the elimination of organic matter in soil. A numeral of procedures upstream of the biocatalysis, e.g., dispersal in solid matrixes, bioavailability, weathering, and abiotic catalysis of pollutants, and downstream, stress, predation, and competition, are acknowledged to oblige the procedure [116]. PCP degraders are ubiquitous at contaminated sites with widespread PCP contamination, but their degradation amounts are relatively low in soil due to low solubility/bioavailability of PCP, poor nutrient level and inappropriate soil redox conditions [69]. The variation of some enzymatic activities and mainly of those partially involved in the contaminant transformation will occur. On the other side, many studies have shown that the addition of supplemental nutrients known as biostimulation procedures, like carbon, nitrogen (C:N) [117]; phosphorous (as phosphates) should mainly increase the rate of xenobiotic compounds degradation such PCP [118]. However, the relationship between nutrient supplementation and microbial degradation of organic contaminants does not appear to be completely straight forward [117].

6.4 The mechanism of microbial remediation of toxic pesticide

Researches on microbial elimination of pesticide residues created in 1940s, and as people reimbursement more consideration to the environment, the research on the elimination procedure and degradation mechanism of organic contaminants has been intensely considered [119]. Bacteria in normal conditions could destroy the pesticide residues, with little cost and environmentally friendly and it would not cause secondary pollution [120]. But the efficacy was moderately slow, and the natural environment was complex and variable, which may disturb the viability and productivity of microbial degradation of pesticides. Consequently, researchers have showed fine studies of bacteria and had a clear considerate of

the degradation mechanism of organic pesticides. Numerous microorganisms have been known in nature, which can disturb the dangerous organic substances in the environment (soil and water) comprising the xenobiotic composites such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and the chlorinated substances approaching polychlorinated biphenyls (PCBs) in due course of time. General of the organochlorines looks to be bio-change, create conjugates with the soil humic matter. Bacterial mineralization of toxic organics logically happening aerobic bacteria decompose both natural and the synthetic hazardous organic materials to harmless CO₂ and water (**Figure 4**). However, mechanism of microbial act in removal of toxic composites is attained by biodegradation and biotransformation of compound toxic chemicals into inoffensive simpler biochemical produces [121].

Microorganisms acclimatize to eliminate “novel artificial compounds” either by using catabolic enzymes they previously possess or by obtaining novel metabolic pathways. Microorganisms break down the complex hydrocarbons in the dangerous waste by via the three general mechanisms-aerobic and anaerobic respiration and fermentation. Aerobic procedure needs satisfactory supply of oxygen, the biodegradation procedure is fast and more complete, and there are no problematic products similar methane and hydrogen sulfide. In anaerobic degradation, for example, there is a sequential, biologically destructive process in which the complex “hydrocarbons” of hazardous wastes are converted into simpler molecules of “carbon dioxide” and “methane.” PCP readily degrades in the environment by chemical, microbiological and photochemical procedures. Degradation in soil is affected by numerous chemical, physical, and biological factors. PCP degrades more quickly in flooded or anaerobic soil than in aerobic moist soil. Numerous pathways of degradation have been studied.

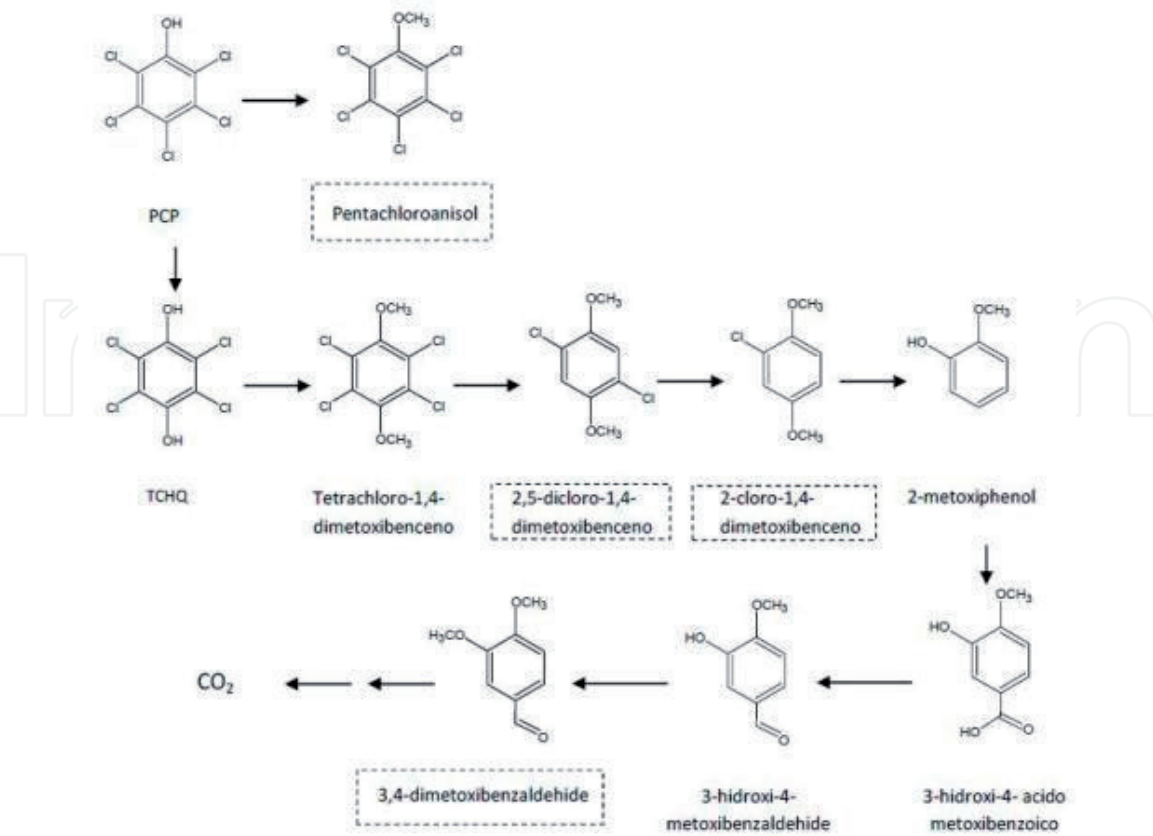


Figure 4.
Biological aspects involved in the degradation of organic pollutants [121].

7. Conclusions

This chapter investigated the effects of PCP soil contamination on microbial diversity, enzymatic activities, microbial biomass, and physicochemical soil characters. In general, the results verified the damaging consequence of PCP on soil activity and variations in soil microbe and genetic variety. PCP negatively affected the intracellular actions of soil microbes and the amount of nitrogen alteration. This may result in the deterioration of soil role and procedures connected to nutrient availability to plants and soil organic matter decomposition and, so, unfavorably affect plant development and health. Moreover, the results presented that the soil fungal community is more sensitive to PCP pollution than the bacterial community. However, enzyme activity can be inhibited at PCP contaminated soil. The denitrification process was significantly reduced by the PCP at a higher rate of PCP, which would further interrupt the nitrogen cycle in soil. Finally, it is necessary to study more details about the effect of PCP accumulation in long-term in soil.

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