The environmental behaviour of polychlorinated phenols and its relevance to cork forest ecosystems: a review

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Pentachlorophenol (PCP) has been used as a herbicide, biocide and preservative worldwide since the 1930s and as a result, extensive and prolonged contamination exists. The environmental impact increases when its many degradation products are taken into consideration. A number of chloroanisols and their related chlorophenols have been found in cork slabs collected from Portuguese oak tree forests before stopper manufacturing, and contamination by PCP and polychlorinated anisole (PCA) has been detected in Canadian forests. It is suggested that the use of polychlorinated phenols, in particular PCP, is thought to be a cause of the cork taint problem in wine, a major socio-economic impact not only for industry but on sensitive and highly biodiverse ecosystems. It also highlights particular issues relating to the regional regulation of potentially toxic chemicals and global economics world wide. To fully understand the impact of contamination sources, the mechanisms responsible for the fate and transport of PCP and its degradation products and assessment of their environmental behaviour is required. This review looks at the current state of knowledge of soil sorption, fate and bioavailability and identifies the challenges of degradation product identification and the contradictory evidence from field and laboratory observations. The need for a systematic evaluation of PCP contamination in relation to cork forest ecosystems and transfer of PCP between trophic levels is emphasised by discrepancies in bioaccumulation and toxicity. This is essential to enable long term management of not only transboundary contaminants, but also the sustainable management of socially and economically important forest ecosystems.

Introduction

The unique physical properties attributed to cork have encouraged its use for sealing bottles, especially for high-quality wines. The frequent occurrence of sensorial alterations in spoiled wines identified as "cork taint" is still a major concern, representing a significant source of economic losses worldwide. This off-flavour, described as mouldy/musty, is commonly related to the chloroanisole family of compounds, especially 2,4,6-trichloroanisole (TCA) and, to a lesser extent, 2,3,4,6-tetrachloroanisole (TeCA) and pentachloroanisole (PCA). The mechanisms leading to the appearance of TCA in wines are difficult to isolate mainly because there are several potential dispersed sources of contamination, which could include the transport and the conservation of either the corks or the bottled wines. In either case, it can be assumed that the direct precursors of chloroanisoles are chlorophenols, 2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP) and pentachlorophenol (PCP), which are converted into less toxic compounds through *O*-methylation reactions mediated by different microbial species. During the last decade the cork industry introduced rigorous quality control in their processes, and have replaced all manufacturing steps with a high risk of contamination by cork taint with safer ones (*e.g.* selective removal of defective cork slabs and removal of chlorination as a water treatment process). Most chlorophenolic products have been banned from use in cellars or during the manufacture of cork stoppers.

The environmental impact increases when its many degradation products are taken into consideration, with chloroanisoles and related chlorophenols found in cork slabs collected from Portuguese oak tree forests before stopper manufacture, and contamination by PCP and PCA detected in Canadian forests.

The cork oak (*Quercus suber*) forest, therefore, may represent a critical scenario for PCP contamination of the food chain. Cork slabs contaminated with polychlorinated phenols, either by direct use or from transboundary contamination where PCP use is still authorised as a pesticide, are likely to be a major cause of the cork taint problem in wine. The focus of this review is to establish the environmental context for forest contamination and the state of knowledge of the role of major environmental variables.

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PCP and its derivatives sodium pentachlorophenate (NaPCP) and pentachlorophenyl laurate (PCPL) have been used worldwide as herbicides, biocides, pesticides and wood preservatives since the 1930s. This extensive use has resulted in the contamination of soils, sediments and waters. Within the European Union (EU), the Marketing and Use Directive (76/769/EEC) and its Ninth Amendment (91/173/EEC) introduced restrictions on PCP's use and at present only professional and industrial use is permitted but under strict conditions. Most EU countries have introduced legislation which prohibits the use of PCP. Production of PCP has ceased within the EU with the remaining countries who use PCP to any great extent (France, Spain, Portugal and the United Kingdom) relying on imports from the United States (US).[1] Production in the US has decreased from 45 million lbs ($\approx$20.4 million kg) in 1983 to approximately 11 million lbs ($\approx$5 million kg).[2,3] Emissions within the UK fell from 538 tonnes to 437 tonnes between 1990 and 2002.[4]

Cork stoppers are produced from the bark of the cork oak *Quercus suber*, an evergreen oak tree found in Portugal, Spain, France, and Italy, with smaller forests in Tunisia, Morocco and Algeria. Spain and Portugal are the world's largest cork producers.[5] Cork is a lightweight material with good thermal insulating properties and is also elastic, compressible and impermeable to gases or liquids[6,7] making it the most suitable material for the manufacture of products which rely on its high compressibility, such as wine stoppers.[8] Although cork is the primary resource of *Quercus suber* forests, they are also used for grazing animals, crop growing and honey production, with estimates that close to 10% of the Tunisian population rely on the cork oak forests for subsistence. Cork forests ecosystems have also been identified as areas rich in biodiversity[9,10] and are home to the Iberian Lynx (*Lynx paradinus*) and the Iberian Eagle (*Aquila adalberti*) two of Europe's most endangered species.

The cork taint phenomena is an earthy, mouldy flavour/aroma that affects 1–5% of bottled wine,[11] with economic losses estimated to be in the order of US $10 billion annually and is predominately associated with chloroanisoles.[12,13] Chloroanisoles are produced within cork *via* fungal methylation of the corresponding chlorophenol. 2,4,6-Trichlorophenol, a PCP transformation product, has been found in trace quantities within wine.[14] It is known that the long lifespan of *Quercus suber*, 160–200 years, allows for the accumulation of polycyclic aromatic hydrocarbons, organochlorine pesticides and other persistent organic pollutants within the bark due to the contaminant's high lipophilicity.[15] An examination of used and unused cork stoppers indicated substantial differences in relative abundance of several classes of organochlorine pesticides, which are probably due to differences in the pesticide usage practices of the various regions in which the oak trees were grown.[15] In other work it was indicated that globally the average amounts of organochlorine pesticides detected in cork were consistent with values found in foods, which suggests that cork is produced to a suitable hygienic quality.[5] Both studies strongly suggest that cork contamination with organochlorine pesticides happens in an early stage of cork stopper production. Apparently the presence of PCP in the forest is a possible explanation of chloroanisole appearance in bottled wine.[13] PCP is often, if not always, associated with cork taint, but PCP contamination of cork may be related to indirect sources of contamination, for example PCP dispersed from nearby located agro-activities or industrial units.

Previous reviews have focused on the transformation of PCP within the environment[16,17] and many studies have shown that a majority of ecosystems are contaminated by PCP, however no research has been carried out in cork forests. This problem is particularly relevant to north Africa because other organochlorine contaminants have already been found in this region.[18–20] Bioavailability, bioaccumulation and toxicity studies have focused on single species, *e.g.* earthworms,[21] but as yet there does not appear to be any significant investigation of PCP bioaccumulation and transfer between various trophic levels.

Degradation products

PCP can degrade into as many as 30 different products depending on the experimental and environmental conditions. The main degradation products are tetrachloro-, trichloro-, and dichlorophenols (TeCP, TCP, DCP),[22] tetrachlorocatechols (TeCC), tetrachloro-, trichloro-, and dichlorohydroquinones (TeCHQ, TCHQ, DCHQ),[23] pentachloroanisole (PCA)[22] and hexachlorobenzene (HCB);[24] polychlorinated diphenylethers and polychlorinated dibenzo-*p*-dioxins are minor products.[25] Degradation of PCP follows first order kinetics with rate constants of 0.16 min^{-1} (at pH 3) and 0.26 min^{-1} (pH 9) in water[26] and 1.3–14.4 min^{-1} (pH 3.9–7.2) in soils.[22] The rate of degradation decreases with decreasing chlorination; the half-lives ($t\frac{1}{2}$) of PCP, 2,4,6-TCP, 2,4-DCP and 4-MCP in water treated with UV irradiation are 132 min, 198 min, 342 min and 3240 min, respectively,[27] possibly as a result of the structural properties of chlorophenols.[28] In river, ground and estuarine water samples the $t\frac{1}{2}$ of PCP was found to be 100 min (ground and estuarine water, pH 7.4 and 8.1, respectively) and 115 min in river water (pH 7.8).[28] This would suggest that the presence of particulate matter in environmental samples, or the type/intensity of irradiation affects degradation rates.

Although the lower chlorinated phenols will always be produced through reductive dechlorination (PCP → TeCP → TCP → DCP), there is confusion as to the products formed and in the order they are formed. The C–Cl bond energies have been calculated as 88.8 kcal mol^{-1} for *ortho*-positions, 87.7 kcal mol^{-1} for *meta*-positions and 87.1 kcal mol^{-1} for the *para*-position, suggesting that the *para*-chlorine is the easiest to dissociate;[29] this study did not detect chlorophenols that had been dechlorinated in the *ortho*-position. However, this is contradicted by other studies that detected 2,3,4,5-TeCP (dechlorinated in the *ortho*-position).[22,25] These studies investigated PCP in water using mercury lamps as UV sources, however the time samples were exposed to irradiation was different; it is possible that differences in results were caused by this. Reductive dechlorination from PCP occurs in soils under anaerobic conditions with transformation rates highly correlated to soil carbon, nitrogen and phosphorous content.[22] Whilst most of the soils studied in this study produced products dechlorinated in the *ortho*-and *para*-positions, one soil (which had the lowest total C content and highest pH) also produced chlorophenols dechlorinated in the *meta*-position, *e.g.* PCP → 2,3,4,6-TeCP → 2,3,6-TCP.

Chlorocatechols and chlorohydroquinones are photo-oxidation products formed *via* dechlorination and the addition of OH radicals. TeCC is formed *via* the dechlorination and addition of OH to the *ortho*-positions; TeCHQ is formed by dechlorination and addition at the *para*-position. TeCC is formed more quickly than TeCHQ suggesting that the *ortho*-chlorines are the easiest to dissociate, again contradictory to the calculated bond energies. These reactions are also pH dependent; at pH 4 similar quantities of TeCC and TeCHQ are produced, however at pH 7 levels of TeCC are greater than TeCHQ26. HCB has been detected as a degradation product of PCP in water[25] and in an organic solvent;[24] although OH bonds are fragile, C–Cl bonds are more easily dissociated. The pathway is thought to be the dechlorination of PCP to TeCP, the free Cl atom then attaches to a PCP molecule which has lost its OH group.[24] It is possible that high levels of HCB (> 81 000 ppb) and low levels of PCP (135 ppb) are due to the fast degradation of PCP.[30]

It is clear that under carefully controlled laboratory conditions PCP is easily degraded and removed from samples, however its persistence in the environment would suggest that sorption to soils, sediments and dissolved and particulate organic matter in waters decreases degradation rates. The apparent lack of agreement in the literature as to the products that are formed, and how they are formed, highlights the difficulty in analysing chlorophenols in environmental samples.

Soil and sediments

The manufacture and use of chlorophenolic compounds has resulted in the contamination of soils[31] and sediments[32] with PCP and lower-chlorinated phenols. The sorption of organic contaminants to soils and sediments can be related to the interaction between the contaminants and soil constituents,[33] such as substrate pH, organic matter content and clay content. Although sorption of chlorophenols is continuous, there is some confusion as to whether it is adsorbed in a smooth fashion[34] or is truly biphasic *i.e.* an initial rapid sorption phase followed by a more prolonged slower phase.[35] However, both studies agree that the majority of PCP is adsorbed rapidly, possibly as a result of (i) PCP entering macro-and mesopores within soils before entering the micropores, which are more difficult to enter or (ii) less available sites being available.[35]

The mobility and fate of PCP can be predicted if both sorption and desorption characteristics are known; these can be described by different isotherms. A number of models have been used, such as the Radke–Prausnitz model,[36] and the triple-layer model,[37] however the most commonly used are the Langmuir model and the Freundlich model.[38,39] Using isotherm results obtained in the laboratory to help our understanding of field soil sorption characteristics should be done with caution as (i) results are only valid under the same conditions as the original experiment,[37] (ii) the field sites may be geochemically too complex to achieve correct sorption values using these relatively simple models[38] and (iii) the Langmuir model has been found to underestimate the amount of PCP adsorbed at concentrations greater than 100 ppm, whilst the Freundlich model overestimated the amount adsorbed at the same concentration.[36]

Sorbate pH is a major factor affecting chlorophenol sorption; as pH increases, sorption decreases.[40,41] This can be attributed to the effects pH has on the chlorophenols and the soil constituents. Tetrachlorophenols and PCP have low $\mathrm{p}K_\mathrm{a}$ values (5.64–4.75, respectively[42]), therefore when the pH $\ll$ $\mathrm{p}K_\mathrm{a}$ chlorophenols are present as the neutral species, when the pH $\gg$ $\mathrm{p}K_\mathrm{a}$ chlorophenols dissociate to their ionic species. Monochloro-, dichloro- and trichlorophenols have higher $\mathrm{p}K_\mathrm{a}$ values than tetrachlorophenols and PCP, therefore they are not influenced by pH to the same extent.[37] As such, tetrachlorophenols and PCP will exist as the ionic species in environmental systems[43] (Table 1).

Neutral chlorophenols are much more readily adsorbed to soils and sediments than ionic chlorophenols;[41] an increase in soil pH causes the soil's overall surface charge to become more negative as more Si–OH sites become dissociated. Electrostatic repulsion between ionic chlorophenols and soils will therefore decrease the amount adsorbed.[37] One study investigated the sorption of monochloro-, dichloro-and trichlorophenols to kaolinite and montmorillonite at two pH values, chosen to represent the pH at which the clays had a neutral surface charge; 4.8 and 9.9 for kaolinite and montmorillonite, respectively. Sorption to kaolinite was greater at pH 4.8 than at 9.9 for two reasons: (i) at pH 9.9 kaolinite has a negative surface charge and (ii) the chlorophenols are present as the ionic species. This combination causes electrostatic repulsions which decreases sorption.[44] It should be noted that although PCP sorption decreases with increasing pH, sorption of ionic PCP is as great as lower-chlorinated phenols; one study found that the sorption co-efficient of ionic PCP in sandy aquifer material was 0.12, whilst for neutral 2,4,6-TCP the value was 0.13.[41]

Table 1 Percentage of ionic chlorophenol present in environmental systems

		pH		
Chlorophenol	$\mathrm{p}K_\mathrm{a}$[a]	4	5	6
2-MCP	8.53	0.0	0.3	2.9
3-MCP	9.13	0.0	0.1	0.7
4-MCP	9.43	0.0	0.0	0.4
2,3-DCP	7.71	0.2	1.9	16.3
2,4-DCP	7.85	0.1	1.4	12.4
2,5-DCP	7.51	0.3	3.0	23.6
2,6-DCP	6.78	1.6	14.2	62.4
3,4-DCP	8.30	0.1	0.5	4.8
3,5-DCP	8.18	0.1	0.7	6.2
2,3,4-TCP	6.97	1.1	9.7	51.7
2,3,5-TCP	6.74	1.8	15.4	64.5
2,3,6-TCP	6.03	8.5	48.3	90.3
2,4,5-TCP	6.72	1.9	16.0	65.6
2,4,6-TCP	6.23	5.6	37.1	85.5
2,3,4,5-TeCP	5.64	18.6	69.6	95.8
2,3,4,6-TeCP	5.22	37.6	85.8	98.4
2,3,5,6-TeCP	5.03	48.3	90.3	98.9
PCP	4.75	64.0	94.7	99.4

[a] $\mathrm{p}K_\mathrm{a}$ taken from Burrows *et al.*[42]

Sheet silicates have been found to be more important sorbents for polar organic herbicides than soil organic matter; soils with a high clay content and a low organic carbon content have greater sorption capacities than soils with low clay content and low organic content.[45] Depending on the 'sheet' configuration, clays can be classified as 1 : 1 or 2 : 1 clays; 1 : 1 clays, such as kaolinite, are the simplest and consist of one alumina and one silica sheet. 2 : 1 clays, such as bentonite and montmorillonite, have a sheet silicate sandwiched between two alumina sheets—this configuration allows the clays to expand in the presence of polar organic molecules and water.[46] Sorption to kaolinite is therefore lower than to bentonite, with *Kf* (Freundlich co-efficient) values of 23.68 and 73.22, respectively, due to the increase in surface area as bentonite expands.[47]

Sorption to 2 : 1 clays is dependent on the cation present within the interlayer spacing—Ca^{2+} containing montmorillonite (Ca-montmorillonite) is known to adsorb less PCP than K-montmorillonite; *Kf* values of 34.19 (at pH 8.37) and 193.69 (pH 8.35), respectively.[48] As the pH values in this study are similar the effects of pH can be excluded, however the explanation for why this might be the case remains unclear; organic contaminants are known to form intercalates in the interlayer spacing,[49] however the formation of chlorophenol-intercalates in montmorillonite clays has not been detected. Chlorophenols have a calculated diameter of 8–10 Å[50] and montmorillonite clays have an interlayer spacing of 9.6–15.5 Å,[46] it would therefore seem probable that chlorophenol-intercalates would form. However, X-ray diffraction (XRD) analysis of Ca-montmorillonite (with or without the presence of PCP) found that there was little difference in the interlayer spacing; suggesting that intercalates do not form, perhaps as a result of competition with water molecules for available sites.[51] K-montmorillonite has an interlayer spacing of approximately 12 Å (smaller than Ca-montmorillonite) which is optimal for the sorption of organic pollutants, as competition with water molecules is avoided.[48,52] Clearly, as no chlorophenol-intercalates have been found in montmorillonite clays when other organic pollutants have, it suggests some other process is involved and further investigation is required. Model clays were used to detail sorption characteristics in the literature, however environmental samples are known to adsorb significantly greater amounts of chlorophenols as clays in soils are weathered, and therefore have a greater sorption capacity[47] and may also be associated with other soil constituents.[45]

The effect of organic matter on sorption of chlorophenols is unclear; organic matter has been found to (i) not affect the sorption of chlorophenols,[53] (ii) decrease the amount bound to substrate,[54] or (iii) aid the sorption of chlorophenols.[40] When organic matter content was varied, 2.4–11.9 mg l^{-1}, at a constant pH it was found that an increase in humic material present did not cause a significant increase in the amount of 2,4,6-TCP and PCP adsorbed.[53] Increasing the amount of humic material has been found to decease the amount of PCP bound to sediments and increased the amount of unbound PCP in water.[54] An increase in the sorption of 2,4,6-TCP, 2,3,4,6-TeCP and PCP has been reported as the organic matter increased.[40] The pH of the sorbents differed (4.8,[53] 6.5[54] and 5.7–8.3[40]), therefore it is possible that pH affected the results; however, the latter study found that for all pH values sorption was greater in soils with high organic matter content. Another study has found that sorption was greater in sandy soils with high organic matter content and low pH than in sandy soils with low organic matter and low pH;[55] this would suggest that organic matter is an important factor affecting chlorophenol sorption regardless of pH.

The organic carbon (OC) content of sediments is known to bind 1.5 times more PCP than dissolved humic matter.[54] The investigation of sediments has found that there is a correlation between OC content and organic pollutants;[20] it has also been reported that soils which contain higher quantities of OC adsorb greater amounts of organic contaminants than soils with little or no OC.[56] However, this may be too simplistic; a recent study worked under the assumption that the organic matter fraction of the soil is solely responsible for the sorption of organic contaminants. This assumption had to be made to determine the effects of OC on sorption but it should be remembered that other studies have shown that other soil constituents affect sorption; it has also been reported in the literature that certain organic pollutants associate with mineralogy and not organic content.[57]

Chlorophenol sorption to soils is thought to be caused by a combination of hydrophobic and electrostatic interactions, although hydrophobic interactions are thought to be stronger as sorption decreases with increasing pH and a corresponding increase in the percentage of ionised species present.[58] Recent investigations of 3,4-DCP, 3,4,5-TCP, 2,3,4,5-TeCP and PCP sorption to soils rich in organic matter (organic carbon content: 10.8–43.6%) found that the relationship between the organic matter distribution co-efficient (K_{OC}) and the octanol–water partition co-efficient (K_{OW}) suggests that hydrophobic bonding is the dominant sorption mechanism. Likewise, by measuring chlorophenol sorption as a function of temperature and calculating the free energy changes (ΔG°) for both neutral and ionised PCP, it was found that hydrophobic bonding is the dominant process for neutral PCP; a more specific bonding process was found to exist for ionised PCP,[39] possibly *via* ligand exchange.[37]

How much contaminant desorbed from the sorbent is determined by a number of factors (pH, clay type), however it is largely determined by the quantity adsorbed in the first place and the length of exposure time. Desorption can be considered to be biphasic; an initial quick release of contaminants is followed by a slower phase.[47] Desorption of contaminants from soils and sediments produces a variety of results, ranging from complete desorption to complete irreversibility, *i.e.* contaminants remain bound to the sorbent; complete desorption occurs when the adsorption co-efficient is low.[59] Increases in the length of time sorbents are exposed to contaminants increases the time it takes to achieve desorption. It has been found that if sorption time increases from 2 to 21 days, the amount desorbed after 2 days decreases from 95% to 68% (for neutral PCP) and 85% to 49% (for ionised PCP); an increase in the desorption time (to 8 days) increased the desorption figure for neutral PCP to 94% whilst only 50% of PCP was desorbed after this period.[39] It can be seen that although it is difficult for ionised chlorophenols to adsorb to sorbents,[41] they are also more resistant to desorption. Clay type is also a factor controlling desorption; an investigation of two artificial soils, one containing kaolinite and the other containing bentonite, found that desorption was greater in the kaolinite soil.[47] Although pH, clay type, clay content and organic matter content all affect chlorophenol sorption and desorption, the interactions between these constituents are often in conflict.

Aquatic environment

Aquatic environments are sensitive to chlorophenols and although levels are decreasing primarily due to decreasing use as new chemicals are introduced to replace them and through the introduction of national legislation banning PCP use,[60] they are still being detected worldwide. 2-MCP, 2,4-DCP, 2,4,6-TCP and PCP have been detected in Portuguese[61] and Japanese[62] river waters and a Chinese study found 2,4-DCP, 2,6-DCP, 2,4,6-TCP, 2,3,4,6-TeCP and PCP at ppb in tap water.[63]

Contamination of waters is caused by the use of chlorophenolic wood preservatives, and the manufacture and agricultural use of PCP, which subsequently degrades into various products. A New Zealand study investigating organochlorine contamination of river and lake water found that one river, which received run-off from a sawmill that had used chlorophenol products over 3 decades, had PCP levels of 3.62 ppb. Other rivers, which were remote from the contamination source, contained levels of 0.01–0.02 ppb,[64] which would suggest atmospheric or possible ground water transport to remote locations. Organochlorines found in the Arctic environment come from distant global sources *via* long-range atmospheric and oceanic transport from lower latitudes,[65] *e.g.* chlorophenols, produced by microbial degradation of PCP, appear in sediments dated as 1940, shortly after the introduction of PCP. Some metabolic by-products of chlorophenols are still being identified in sediment cores, suggesting that fresh applications of legal and illegal chlorinated pesticides are adding to Arctic contamination. In China, an investigation of river and pond sediments found that PCP levels were higher in pond sediments than river sediments (37.5 ppb and 5.45 ppb, respectively); this study also found that highly industrialised areas had lower PCP values than less industrialised areas (2.20 ppb and 37.5 ppb, respectively) which would suggest agricultural sources of contamination.[66] Ponds are closed systems and, unlike rivers, contaminants cannot be removed easily, and therefore levels increase; PCP within river waters has been found to be removed through outflow and biodegradation.[67]

One method of determining if a contaminant poses a risk to the environment is to calculate the ratio of the predicted environmental concentration (PEC) to predicted no-effect concentration (PNEC); if the value is >1 then a pollutant can be considered to pose a risk. The PCP–PEC value for marine and river water in the North Sea region has been reported as 0.07 $\mu g\ l^{-1}$ in the period 1983–1997. The calculated PEC : PNEC ratio for waters and sediments in this region were found to be <1,[68] therefore no risk can be considered. However, there are two locations where the PEC : PNEC ratio of sediments was found to be >1—northern France and the Netherlands. Industry in northern France is reported to use approximately 10 times less NaPCP than southern France, however the PEC : PNEC ratio for northern France was 1.36, and for southern France it was 0.041. The waters of northern France contain more particulate matter than southern France and it is thought that reduced rates of photodegradation in northern waters is responsible for the high PEC : PNEC ratio.[1] Although a national ban on the use of PCP exists in the Netherlands the PEC : PNEC ratio of sediments was larger than 1; this is thought to be partly due to historic use (chlorophenols are lipophilic and are strongly bound to sediments[69]) and partly due to rivers that travel through countries where PCP is still in use and is subsequently transported downstream, *e.g.* the River Meuse which originates in France and reaches the North Sea in the Netherlands.

Bioaccumulation and toxicity

Bioaccumulation and bioavailability are closely linked; bioavailability can be defined as the portion of chemical that has not been adsorbed to soil and is available for interactions with soil biota.[70] Soils and sediments with a high organic matter content have lower bioavailable fractions than those with a low organic matter content.[69,71] Competitive sorption between humic acids and chlorinated organic contaminants is suggested to be able to increase the mobility and bioavailable fraction of contaminants and may explain differences between sorption and desorption isotherms.[72] It is possible to correlate sorption and desorption isotherms with the bioaccumulation of various chlorophenols; the greatest bioaccumulation occurring if there are differences between the sorption and desorption isotherm.[73] Neutral chlorophenols are bound to soils more easily than ionised chlorophenols and are also more mobile due to the ease of desorption, therefore bioaccumulation increases at low pH values.[74,75]

Chlorophenols are bioaccumulative in humans, and aquatic[76] and terrestrial[77] organisms. A 1998 environmental survey in Germany found the mean PCP concentration in human urine was 1.0 ppb, which was down from 2.7 ppb in 1992. 4-MCP was found at the highest concentrations in all samples ($n = 692$) with a mean concentration of 4.9 ppb, partly as a result of it being a metabolite of chlorobenzene, which is used as a herbicide.[78] Analysis of sawmill workers in Taiwan found urine levels of 0.08 ppb, 0.13 ppb, 1.56 ppb and 0.6 ppb for PCP, 2,3,4-TCP, 2,4-DCP and 2-MCP, respectively.[79] Breast milk sampled from first time mothers in China contained an average of 2.15 ppb[66] PCP and levels of 1.27 ppb have been detected in the urine samples of American pre-school children.[80] The use of PCP and chlorophenols has been prohibited in Germany and Taiwan since 1989 and in China since 1987; the detection of PCP and its transformation products many years after this highlights their persistence in the environment. Repeated human exposure *via* dermal exposure, inhalation and ingestion, is known to cause headaches, drowsiness and numbness of the limbs.[81]

Contaminants can be accumulated without being toxic; toxicity of chlorophenols is dependent on the degree of chlorination, position of the chlorine atoms, species and pH.

Table 2 The toxicity values shown as EC$_{50}$ and LC$_{50}$ of various chlorophenols to an algae (*Pseudokirchneriella subcapitata*), bacteria (*Pseudomonas flourescens*) and an earthworm (*Eisenia fetida*)[a]

Chlorophenol	*Pseudokirchneriella subcapitata*[86] EC$_{50}$/mmol l^{-1}	*Pseudomonas flourescens*[82] EC$_{50}$/mmol l^{-1}	*Eisenia fetida*[77] LC$_{50}$/mmol cm^{-2}
Phenol	0.27552	—	0.00036
2-MCP	0.15985	0.95	0.000023
3-MCP	—	0.27	0.000019
4-MCP	0.16242	0.095	0.000026
2,3-DCP	0.01859	0.33	0.000015
2,4-DCP	0.02577	0.09	0.000017
2,5-DCP	—	0.17	—
2,6-DCP	—	0.48	0.000015
3,4-DCP	—	0.54	0.000015
3,5-DCP	—	0.018	0.0000074
2,3,4-TCP	—	0.015	0.0000071
2,3,5-TCP	—	0.025	—
2,3,6-TCP	—	0.092	—
2,4,5-TCP	—	0.057	0.0000071
2,4,6-TCP	0.00406	0.026	0.0000061
3,4,5-TCP	—	0.0095	—
2,3,4,6-TeCP	0.00031	—	0.0000043
PCP	0.00002	—	—

[a] — not investigated.

Table 2 lists the 50% effective concentration (EC$_{50}$) and 50% lethal concentration (LC$_{50}$) for a bacteria, an algae and an earthworm for various chlorophenols. As can be seen from Table 2 and Fig. 1, the general trend for toxicity is PCP > TeCP > TCP > DCP > MCP > phenol. However, a study of the bacteria *Pseudomonas flourescens* found that the position of the chlorine atom is important. This study found that monochlorophenols chlorinated in the *para*-position were more toxic than if the chlorophenol was chlorinate in the *ortho*-position; EC$_{50}$ values of 0.095 mmol l^{-1} and 0.950 mmol l^{-1} for 4-MCP (*para*-) and 2-MCP (*ortho*-), respectively. This is the same for dichlorophenols (3,4-DCP – 0.054 mmol l^{-1}; 2,3-DCP – 0.33 mmol l^{-1}) and trichlorophenols (2,3,6-TCP – 0.092 mmol l^{-1}; 3,4,5-TCP – 0.0095 mmol l^{-1}), the result being that some dichlorophenols are more toxic than trichlorophenols.[82] Toxicity of 'PCP' has been found to increase and decrease as a result of the formation and degradation of transformation products.[83]

It has also been found that as pH increases the toxicity of PCP to soil microbiota increases; *e.g.* ED$_{10}$ values of 0.338 mmol kg^{-1} and 0.004 mmol kg^{-1} for pH values 3.45 and 7.79, respectively.[33] Analysis of PCP toxicity to *Eisenia fetida* also found that ionised PCP is more toxic than neutral PCP; at a concentration of 50 ppm (PCP in soil) the earthworms gained an average of 0.161 g of biomass at pH 4.6 and only 0.078 g at pH 7.4.[84] At low PCP concentrations in sandy soils (< 10 ppb, sand content 79%, pH 7.0), soil microbial content was found to contain less than half the number of species of microalgae and cyanobacteria than uncontaminated soils.[85] It is suspected that PCP targets photosynthetic reactions, as PCP is more

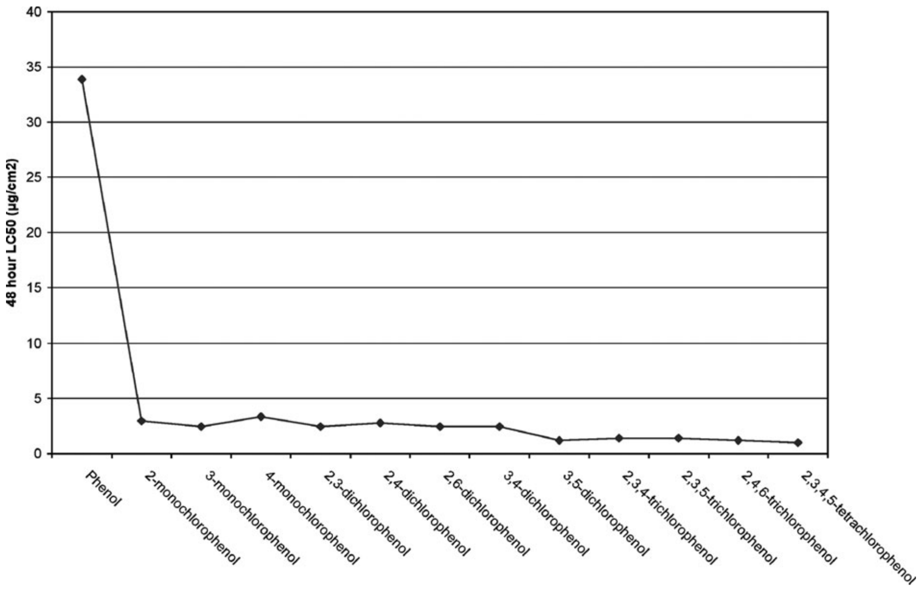

Fig. 1 48 h LC$_{50}$ of various chlorophenols to earthworms, *Eisenia fetida*, using paper contact method. Based on Miyazaki *et al.*[77]

than three times toxic to dissolved oxygen production than to microbial growth.[86]

Bioremediation

The degradation of PCP and the formation of less toxic transformation products by filamentous fungi has been the focus of bioremediation efforts. The ligninolytic enzymes of white-rot fungi have broad substrate specificity and have been implicated in the transformation and mineralization of organopollutants with structural similarities to lignin.[87] White-rot fungi occur naturally on wood[88] and the most commonly investigated species are *Phanerochaete chrysosporium* and *Trametes versicolor* because they can degrade PCP, mainly through reductive dechlorination reactions that presumably lead to its complete mineralisation to CO_2.[89] PCP degradation in some cases may result in the production of pentachloroanisole (PCA),[88,90,91] which is seen as a detoxifying step, as PCA is less toxic than PCP.[92] PCP co-polymerization with low-molecular weight aromatic compounds produced during decay of lignin would reduce PCP bioavailability. This was suggested after the observation of *in vitro* polymerization of PCP to ferulic acid by enzymes produced by *P. chrysosporium*.[90] PCP transformation in soils after inoculation with *P. chrysosporium* has lead to a decrease of the PCP level, but apparently not in the overall toxicity, suggesting that the formation of toxic transformation products occur during PCP bioremediation.[83]

PCP degradation is not an exclusive feature of white-rot fungi, soft rot species are frequently found in organochlorine pesticide contaminated soils[93] and some species, for example filamentous fungi ascomycetes isolated from cork slabs,[94,95] have also shown the ability to degrade some chlorinated phenols.[13,96,97] *Chrysonilia sitophila* was observed to degrade 2,4,6-trichlorophenol and did not lead to significant production of the corresponding anisole.[96] This fungal species was reported to express lignin peroxidase activity.[98] The potential of cork colonising species for PCP bioremediation is only now being explored. The ability of these species to grow in a cork based media, which is enriched in lignin and suberin phenolic-polymers,[99] may suggest active PCP bioremediation. Additionally, soft rot fungi ascomycetes ensure efficient degradation of lignin enriched composites in soil, where high moisture and reduced oxygen conditions prohibit the activity of other fungi.[100] Some common ascomycetes genera *e.g. Trichoderma, Aspergillus* and *Penicillium*, are capable of mineralizing synthetic lignins to CO_2; moreover, the transformation is not limited to methoxyls, but also includes propanoid groups and even aromatic rings.[89]

Conclusions

Current and historical use of PCP is an environmental concern. Transportation of man-made chemicals, *via* air or water, results in extensive contamination of all ecosystems. Through direct use of chlorophenols as biocides, preservatives and pesticides, in industry and agriculture has led to contamination of soils, sediments, waters and through wine production, has resulted in exposure of higher levels of the food chain. Despite the number of environmentally relevant studies of PCP and its degradation products, there is a significant degree of uncertainty in environmental behaviour, associated bioavailability and accumulation. The complexity of degradation pathways provides a challenge for chemical analysis and many of the conclusions regarding fate/transport are obscured by incomplete understanding of these pathways. The comparison of laboratory and field behaviour produces often conflicting conclusions regarding the role of organic matter and pH and the influence of the properties of solids such as the clay minerals. This has a knock on effect in describing the dynamics of sorption–desorption to solid phases and their role in models for dispersion prediction. Forests are commonly perceived as non-polluted ecosystems and have been neglected in terms of wider environmental management. However, whilst the transfer of PCP related contamination has been indirectly identified and external factors are a probable cause, the levels and variation of PCP and its degradation products in cork forest ecosystems have yet to be systematically investigated and it is clear that without a detailed mechanistic understanding, the variation in environmental variables will introduce an unacceptable uncertainty to our model of fate and risk. This is especially true in northern African forests where other organochlorine contaminants have been identified and the importing of cork from this source into the EU potentially short circuits regulatory restrictions. In line with evolving regulatory approaches globally to manage potential transboundary pollution issues, this review highlights the need for a ‘multipurpose’ monitoring strategy, linking urban, rural and global sites to systematically examine chlorophenol contamination within cork forests and the transfer between trophic levels. A case study which identifies a strong link between scientific understanding and policy implementation.

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