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Retention and Extractability of Phenol, Cresol, and Dichlorophenol Exposed to Two Surface Soils in the Presence of Horseradish Peroxidase Enzyme

FANGXIANG XU AND ALOK BHANDARI*

Department of Civil Engineering, Kansas State University, Manhattan, Kansas 66506-5000

The retention of phenol, *o*-cresol, 2,4-dichlorophenol (DCP), and their peroxidase-catalyzed polymerization products was evaluated on two surface soils. The extractability of the parent solutes and their polymerization products was also investigated. ^{14}C-Labeled radioisotopes were used to quantify the contaminant retained on soil as water-extractable, methanol-extractable, humic/fulvic (HA/FA) acid-bound, and soil/humin bound. Between 2 and 20% of the solute retained on soil after a 7-day contact period remained bound to the HA/FA and soil/humin components in unamended soils; in the presence of peroxidase this amount was as high as 40−75%. The alkali-extractable HA/FA component contained the largest fraction of radioactivity in peroxidase-amended soils. Whereas the soil organic matter content was the predominant factor controlling the extent of sorption of the parent phenols, the clay content and particle surface area appeared to contribute to the retention of the polymerization products. High molecular weight oligomers produced during peroxidase-mediated polymerization of phenols associate strongly with soil components and are likely incorporated into the soil organic matter via oxidative coupling reactions.

KEYWORDS: Soil organic matter; enzyme; oxidative coupling; polymerization; humification

INTRODUCTION

Phenols are common soil and groundwater pollutants. These chemicals are classified as priority pollutants due to their toxic health effects at very low concentrations (*1*). Phenols in soil and groundwater are derivatives of natural bio-geochemical processes or have their origins in anthropogenic materials such as pesticide mixtures. The type and extent of substitution on the aromatic ring often control the physical and chemical properties of these chemicals and can influence their mobility and environmental fate in soils and groundwater (*2*). For example, the hydroxyl (−OH) substituent in phenols imparts greater solubility and reactivity to these chemicals. The presence of other substituents (e.g., −Cl, $-NH_2$, $-NO_2$, and $-C_xH_y$) on the phenol can further alter the solubility and reactivity of these compounds. Soils contaminated with anthropogenic phenols are of specific environmental concern because of the ecological risk associated with their high toxicity and relatively high mobility in the soil and groundwater environment.

Several researchers have studied the sorption−desorption of phenolic chemicals in soil−water and sediment−water systems (*3*−*8*). Phenols have been found to possess relatively high mobilities in the aquifer environment because their sorption to mineral surfaces is usually minimal (*9*). Soil organic matter (SOM) is seen as a dominant factor influencing the fate of organic contaminants in soils and aquifer material (*10*−*13*). Solution pH and the number and type of substituent groups on the phenol ring have also been shown to impact the sorption−desorption properties of these chemicals (*2*, *14*).

Desorption of phenols can be hysteretic or irreversible in nonsterile soils. Sabbah and Rebhun found strong desorption hysteresis for 2,4,6-trichlorophenol (TCP) in organic-rich soils; no hysteresis was observed in pure clay mineral or in soil with high clay and low organic contents (*8*). Sorption irreversibility was also observed during adsorption−desorption of 4-chlorophenol (CP), 2,4-dichlorophenol (DCP), and 2,4-diphenoxyacetic acid (2,4-D) in a variety of organic matrices (*6*). Sorption irreversibility was lower for phenols with a greater number of −Cl substitutents. Isaacson and Frink used a continuous-flow stirred cell to study sorption of phenol, 2-CP, and 2,4-DCP in sediment fractions and found that in some cases up to 90% of the solute was irreversibly bound (*3*). While extracting pentachlorophenol (PCP) with an ethanol−water mixture, Khodadoust and co-workers found that a fraction of PCP remained nonextractable, irrespective of the ethanol−water ratio used (*15*).

Several researchers have attributed the irreversible binding of phenols to oxidative coupling reactions that result in covalent bond formation between the phenolic chemical and SOM (*6*, *15*−*17*). In the soil environment, these reactions are believed to be catalyzed by soil enzymes (*18*) and transition metal oxides (*19*−*21*). Studies have shown the ability of soil enzymes, such as peroxidases and polyphenol oxidases, to polymerize phenols by cross-coupling following oxidation of the phenol by the enzyme (*22*, *23*). In the presence of hydrogen peroxide or

* Corresponding author [telephone (785) 532-1578; fax (785) 532-7717; e-mail bhandari@ksu.edu].

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Table 1. Selected Soil Properties

	agricultural soil	forest soil
classification	Haynie Series coarse-silty, mixed, calcareous, mesic Mollic Udufluents	
particle size analysis		
% sand	44	56
% silt	42	38
% clay	14	6
organic matter content (Walkley–Black method) (%)	2.5	3.7
cation exchange capacity (mequiv/100 g)	9.7	10.9
N_2 BET surface area (single point at $P/P_0 = 0.30$) (m^2/g)	13.36	4.17
whole soil elemental analysis		
% carbon	1.02	1.50
% hydrogen	0.31	0.27
% oxygen	2.94	2.58
% nitrogen	0.09	0.13
% sulfur	0.02	0.03
% ash	95.44	95.98

molecular oxygen, these enzymes oxidize phenolic chemicals to reactive free radicals or quinones that subsequently react with other phenols in solution to form cross-coupled products or oligomers. The rate of oxidative coupling of phenols is often strongly related to the type of substituent groups (electron-donating or electron-withdrawing substituents) present on the aromatic ring (*24*).

Enzyme-mediated oxidative coupling reactions have been found to result in the binding of phenols to dissolved organic matter (*25*−*27*). Incorporation of phenols into natural organic matter macromolecules during enzymatic polymerization can result in detoxification of the parent chemical (*28*−*30*). The low solubility of the oligomers produced and the fortuitous dehalogenation of chlorinated phenols during cross-coupling have been suggested to result in toxicity reductions (*31*, *32*). Polymerized products and phenols bound to natural organic matter are believed to be less available for subsequent microbial uptake (*33*, *34*). Enzyme-catalyzed bound-residue formation has been suggested as a cleanup strategy for soils containing phenolic contaminants (*35*).

In a recent study, we presented the sorption−desorption characteristics of phenol, *o*-cresol, DCP, and 1-naphthol in two sandy loams in the presence and absence of horseradish peroxidase (*36*). In this paper we report the extents of binding and extractability of phenols and their polymerized products exposed to the two soils.

MATERIALS AND METHODS

Soils. Two surface soils were selected for this study. These soils were collected aseptically from the Ap horizon of an agricultural field and the A horizon of an adjacent forested area near Manhattan, KS. Both soils belonged to the Haynie series and were classified as coarse-silty, mixed, calcareous, mesic Mollic Udufluents. The soils were crushed and sieved to pass through a 500-μm screen and sterilized using a multiple autoclaving procedure. The soils were prewashed with a synthetic groundwater solution (pH 7 phosphate buffer + 500 mg/L NaN_3 and 18 mM ionic strength) to remove easily leachable soil organic matter and avoid a three-phase (soil, water, and dissolved organic matter) system. Autoclaved and prewashed soils were stored at − 4°C until further use. Selected properties of the soils are summarized in **Table 1**. The mineralogical composition of the Haynie soils consisted of 40% montmorillonite, 20% kaolinite, 20% mica, 10% quartz, and 10% montmorillonite-mica. Elemental analyses and surface area measurements on the bulk soils were performed by Huffman Laboratories, Inc. (Golden, CO) and Micromeritics Instrument Corp., (Norcross, GA), respectively.

Table 2. Selected Properties of the Target Chemicals

chemical property	phenol	*o*-cresol	2,4-DCP
mol wt (g/mol)	94.1	108.2	163.0
aqueous solubility (g/L)	80	25	4.5
log K_{OW}	1.46	1.95	3.20
pK_a	9.8	10.2	7.9

Chemicals. Target chemicals used in this study included three phenols—phenol, *o*-cresol, and 2,4-DCP. Selected properties of these chemicals are summarized in **Table 2**. Uniformly ring-^{14}C-labeled isotopes were used to facilitate tracking of the solute among various soil fractions. The radiochemical purity and specific activity for phenol were >98% and 14.3 mCi/mmol, respectively. The corresponding numbers for cresol and DCP were >95% and 4.8 mCi/mmol and >95% and 20.9 mCi/mmol, respectively. Radioisotopes and nonlabeled chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. All stock solutions were prepared in methanol, and working solutions were prepared in synthetic groundwater (GW). This solution contained 500 mg/L NaN_3 to inhibit bioactivity. Working solutions were spiked with enough radioisotope to result in an activity equivalent to 8000 disintegrations per minute (dpm) per milliliter of solution. To avoid cosolvency effects caused by the presence of methanol, the volume of stock solutions added to GW was restricted to <0.1% v/v. A Beckman 6500 liquid scintillation counter (LSC) with quench and luminescence corrections was used to quantify radioactivity.

Horseradish peroxidase (HRP, type II, RZ: 2.2), hydrogen peroxide (30%), and sodium azide were purchased from Sigma Chemical Co. and used without further purification. Each enzyme activity unit (AU) of peroxidase used in this study was defined as the amount of enzyme forming 1.0 mg of purpurogallin from pyrogallol in 20 s at pH 6.0 and 20 °C.

Sorption. Five grams of soil (dry weight) was mixed with ~14 mL of solution in glass centrifuge tube reactors. Initial aqueous concentrations (C_0) of the target chemicals were 5, 50, and 500 μM. The treatments evaluated included (a) solution only, (b) solution plus soil, and (c) solution plus soil plus peroxidase plus H_2O_2. Triplicate sets of tubes were prepared for each concentration plus treatment combination. No Fenton reagent activity was observed to occur at the H_2O_2 concentrations used (*36*). Preliminary studies also showed that H_2O_2 or enzyme added separately did not affect the sorption process. Although some researchers have observed sodium azide to act as a mechanism-based inactivator of horseradish peroxidase (*37*), our studies showed nearly complete transformation of the parent phenols in aqueous systems at the concentrations of enzyme, H_2O_2, and phenols used for sorption experiments.

Tubes with enzyme received an HRP dose of 2 AU/mL. HRP addition was always accompanied with the addition of precise amounts of H_2O_2 to produce solution concentrations equimolar to the sum of molar concentration of all phenols in solution. The tubes were allowed to equilibrate for 7 days at room temperature (22 ± 1 °C) in an end-over-end tumbler. After 7 days, the contents of the tubes were centrifuged for 45 min at 550*g*, and the aqueous phase solute concentration was determined by measuring the radioactivity in a 250-μL aliquot of the supernatant. The solid phase concentration of the target chemical was determined by subtracting the mass of solute in the aqueous phase from the total mass added to each tube.

Extractions. Supernatant from each centrifuge tube was removed and replaced by uncontaminated GW solution. The mass of the tube and its contents was recorded after each transfer and used to determine the exact volume of solution removed. The tubes were re-placed in the tumbler to mix for 24 h at 22 ± 1 °C. Tubes were removed on the following day, their contents were centrifuged, and the supernatant was sampled for aqueous phase radioactivity resulting from desorption of the solute. The remaining supernatant was removed, and the tubes were refilled with uncontaminated GW. This "fill-and-draw" extraction procedure was repeated until the radioactivity measured in the supernatant fell below the background level of 50 dpm.

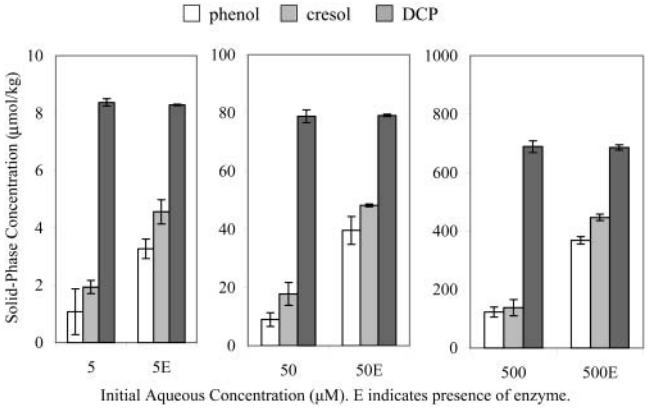

Figure 1. Sorption of phenol, cresol, and DCP, and retention of their peroxidase-catalyzed polymerization products on forest soil.

Water extraction was followed by a similar fill-and-draw sequential extraction procedure with an organic solvent. On the basis of a preliminary study that investigated extraction efficiencies using six different solvents, we selected methanol as the extraction fluid for the three phenols (*38*). The solvent extraction procedure was repeated until the activity measured in the supernatant fell below 50 dpm.

Each water- and methanol-extracted soil sample was divided into two subsamples. One subsample was extracted with 0.1 N NaOH under a nitrogen atmosphere. The fill-and-draw procedure was also used for the alkali extractions. However, the supernatant was not sampled for radioactivity as the humic and fulvic acids effectively shielded the β-particles emitted from the radioisotope and prevented detection by the LSC. Sequential alkali extractions were continued until the supernatant appeared visibly clear, indicating that all humic and fulvic acids had been extracted. Next, both soil subsamples were combusted at 925 °C in a Biological Oxidizer OX-500 (R. J. Harvey Instrument Co.). Combustion resulted in the conversion of the ^{14}C-labeled target chemical in the soil to $^{14}CO_2$. The $^{14}CO_2$ was captured in OX-161 carbon-14 cocktail (R. J. Harvey Instrument Co.) and quantified on the LSC. The amount of $^{14}CO_2$ produced during combustion was used to determine the amount of target solute associated with the soil before and after alkali extraction.

RESULTS AND DISCUSSION

Peroxidase addition can significantly alter the phase distribution of phenolic chemicals between water and soil (*36*). Removal of phenols from water in the presence of peroxidase results from a combination of processes including partitioning and sorption of the polymers produced and the direct cross-coupling of the parent phenol or the polymers with SOM macromolecules. Results presented in this paper describe the impact of peroxidase addition on the extractability of the phenols and their oligomers retained on two surface soils during a 7-day contact period. In this paper, the term "sorption" is used to describe partitioning of the parent phenols on soil in the absence of enzyme and the term "retention" is used to describe the mass transfer and binding of polymerization products in enzyme-amended soils.

Figure 1 illustrates the effect of peroxidase addition on the sorption and retention of parent phenols and their polymerized products on the forest soil. The figure graphically presents the equilibrium solid phase concentrations achieved when the soil was contacted with 5, 50, and 500 μM solutions of the respective solutes in the absence and presence of enzyme. Although sorption of cresol to the forest soil was only marginally greater than that of phenol, DCP sorbed to a significantly greater extent than the other phenols. The sorption behavior of the three phenols appeared to be controlled by their chemical properties, specifically their aqueous solubility and octanol−water partition coefficient (**Table 2**). DCP, with the lowest solubility and highest K_{OW}, sorbed to the greatest extent.

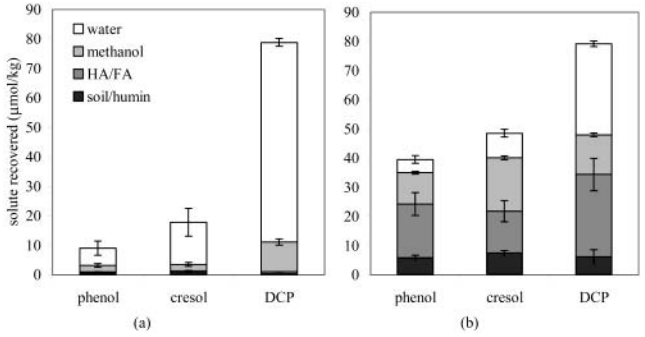

Figure 2. Recovery of (a) phenol, cresol, and DCP, and (b) their respective polymerization products contacted at $C_0 = 50$ μM with the forest soil.

Polymerization products formed from phenol and cresol in the presence of enzyme were retained on the forest soil more readily than the parent solutes. A 3.0−4.4-fold increase in retention was observed for phenol polymerization products as compared to the solid phase concentrations of the parent phenol. In the case of cresol, a 2.4−3.3-fold enhancement in retention was observed. Although peroxidase-mediated polymerization of DCP is extensively documented (*22*−*24*, *26*, *29*, *34*) and was visually observed (from browning of the solution) in our study, retention of DCP polymerization products on the forest soil did not appear to be significantly different from the sorption of the parent solute. We believe that polymerization of phenol and cresol resulted in products that were significantly more hydrophobic and less soluble compared to the parent solutes, whereas the solubility reduction of the oligomers formed from DCP was not significant enough to alter its phase distribution behavior between water and soil. In the forest soil, therefore, although peroxidase addition did result in DCP polymerization, no apparent enhancement in DCP removal from the aqueous phase was seen to occur. Phenols with electron-withdrawing substituents such as −Cl have also been shown to polymerize less efficiently during peroxidase-mediated oxidative coupling reactions (*24*). It is possible, therefore, that DCP formed smaller or fewer oligomers that failed to associate favorably with the forest soil as compared to the parent solute, thereby manifesting an overall sorption behavior not too different from that of the parent solute.

Extractability of the solutes and their polymerization products retained on the forest soil was evaluated using a groundwater solution (18 mM ionic strength), methanol, and 0.1 N NaOH. The recovery of phenol, cresol, DCP, and their polymerization products from the forest soil exposed to an initial solute concentration, C_0, of 50 μM is illustrated graphically in **Figure 2**. Panels a and b of **Figure 2** present solute recoveries in the three extracts and the residual solute in the soil/humin fraction in the absence and presence of peroxidase, respectively. In the absence of enzyme, nearly all of the sorbed solute (phenol, cresol, or DCP) was recovered during water and methanol extractions. Recoveries in the water and solvent extracts were between 90 and 93% for phenol and cresol and as much as 98% for DCP. Approximately 2.8−5.7% of the sorbed phenol and cresol was extracted in the alkali solution. Incomplete recoveries of solutes (in the absence of enzyme) have been attributed to entrapment of the chemicals in soil micropores (*39*). It is likely that the smaller molecular dimensions of phenol and cresol as compared to DCP allowed greater penetration of these molecules into the soil matrix and consequently greater entrapment in intraparticle or intraorganic matter sites.

The impact of peroxidase addition on the retention and extractability of polymerization products was also evaluated.

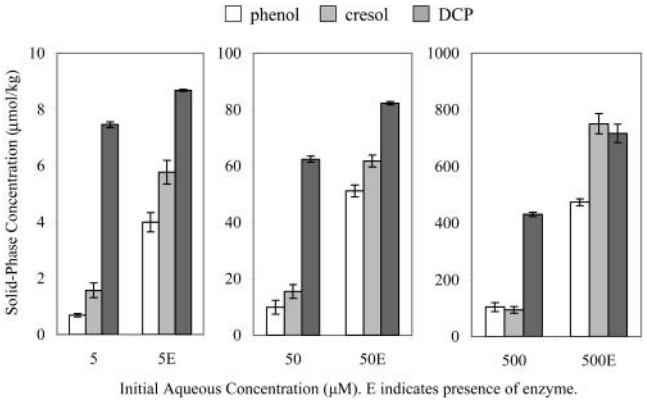

Figure 3. (a) Sorption of phenol, cresol, and DCP, and (b) retention of their peroxidase-catalyzed polymerization products on agricultural soil.

A comparison of panels a and b of **Figure 2** shows that in addition to enhancement in retention, the extractability behaviors of the polymerization products were also significantly different from those of the parent solutes. Peroxidase addition to soil solution appeared to result in significant attenuation of solute extractability. This was true even in the case of DCP, when enzyme addition did not produce any significant increase in the retention of the polymerization products on the forest soil.

Recovery of the polymerized products in the water and solvent extracts was significantly less than that observed for the parent solutes. Only 35−40% of the phenol polymerization products were extracted by water and solvent, whereas recoveries for cresol and DCP polymerization products were 55−64 and 52−60%, respectively. Enhancements in the HA/FA and soil/humin bound fractions of the phenols were more pronounced than increases in overall sorption. Peroxidase addition to forest soil resulted in soil/humin bound fractions of phenol, *o*-cresol, and DCP polymerization products that were 21, 16, and 17 times those of the parent solutes in the absence of enzyme, respectively. Binding enhancements were even larger in the HA/FA fractions: 52, 47, and 65 times more phenol, cresol, and DCP polymerization products were associated with the alkali-extractable SOM in enzyme-amended forest soil as compared to the parent solutes in unamended soil.

Even in the case of DCP, when enzyme addition did not increase overall solute retention, the extractability of the oligomers was significantly reduced. Approximately 7−10 and 33−36% of the DCP oligomers retained on the forest soil were strongly associated with the soil/humin and HA/FA components, respectively. Thus, although no significant increase in the overall retention of DCP oligomers was seen on forest soil, the differences in extractability in the absence and presence of peroxidase served as confirmation of our visual observations of DCP polymerization.

Sorption of phenol, cresol, and DCP and the retention of their polymerization products on the agricultural soil are graphically illustrated in **Figure 3**. Although both agricultural and forest soils had identical taxonomic classifications, the agricultural soil differed significantly from the forest soil in its organic matter and clay contents. In the context of sorption of solutes from aqueous solutions, the lower organic matter content of the agricultural soil appeared to be well compensated by its higher clay content. The significantly greater N_2 BET surface area of the agricultural soil was likely a result of its larger clay fraction.

The sorption behavior of the parent solutes appeared to be governed by the organic matter contents of the two soils—phenol, cresol, and DCP sorbed to a lesser extent on the agricultural soil than on the forest soil. However, the agricultural

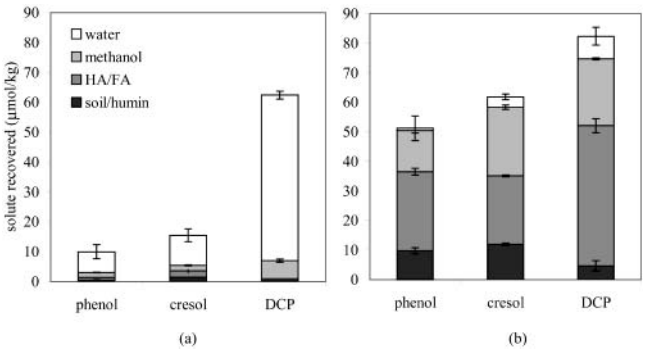

Figure 4. Recovery of (a) phenol, cresol, and DCP, and (b) their respective polymerization products contacted at $C_0 = 50$ μM with the agricultural soil.

soil retained a greater amount of the polymerization products than the forest soil. The enhancement in retention of polymerization products as compared to the parent solutes was also significantly more pronounced in the agricultural soil than in the forest soil. Furthermore, unlike in the forest soil, enhanced retention was also observed for DCP oligomers on the agricultural soil. These differences exhibited by the agricultural soil seem to be related to its higher clay content and surface area. Although SOM content appears to have controlled the extent of sorption of the parent solutes, it was the available surface area that seemed to govern the phase distribution of the polymerization products in the surface soils. The higher surface area available in the agricultural soil provided an expansive domain for the sorption and binding of the large molecular weight polymerization products. In the case of DCP, the availability of a large inorganic sorbent surface allowed for greater mass transfer of the DCP polymerization products in the agricultural soil than in the forest soil.

Figure 4 shows the extractability results for the agricultural soil for $C_0 = 50$ μM. Overall sorption of the parent solutes was smaller, whereas the retention of polymerization products was generally greater in the agricultural soil than in the forest soil. However, as seen in **Figure 4b**, significantly larger amounts of polymerization products were found in the alkali-extractable humic/fulvic acid and the soil/humin components in the agricultural soil than in the forest soil. Large quantities of polymerization products retained on forest and agricultural soils were recovered in the HA/FA fraction. Several researchers have observed that the addition of peroxidase and similar enzymes to solutions containing dissolved organic matter can result in the cross-coupling of phenols and phenolic polymers to dissolved organic matter (*26*, *27*). It is likely that these mechanisms were operative in our soil solutions and helped transform the parent solutes and their oligomers to bound residues by catalyzing the formation of covalent bonds between the contaminants and the phenolic moieties of the SOM macromolecules. It is also possible that some oligomers produced during cross-coupling dropped out of solution due to their large size and low solubility and adhered strongly but non-covalently to the soil particles (*40*). The structural similarity of these oligomers to humic and fulvic acids (*41*) may have resulted in their recovery during base extraction and their operational classification as "HA/FA-bound".

Peroxidase addition also resulted in large "soil/humin bound" fractions of all polymerization products. This fraction may have included solute molecules that were directly bound to the humin fraction of the SOM or oligomers associated with mineral surfaces that were not soluble in alkali. It is also likely that the soil/humin bound fraction included contaminant that was not

bound to soil but physically trapped or sequestered in micropores associated with the mineral and SOM domains (*39*).

Results from our experiments lead us to believe that peroxidase-mediated oxidative coupling reactions immobilize phenolic contaminants in the soil solution in two ways. First, phenol molecules are polymerized into a variety of high molecular weight, low-solubility oligomers that fall out of solution and associate strongly with particle surfaces in soil. Second, phenolic free radicals and quinones generated in the presence of HRP mediate the direct oxidative coupling or covalent bond formation between the phenolic oligomers or parent solutes and the SOM macromolecules. This strong binding of phenolic oligomers with SOM and mineral surfaces in HRP-amended soils results in low extraction efficiency in water and greater association with the HA/FA and soil/humin components as compared to the parent solutes in unamended soils. Furthermore, the greatest enhancement in bound residue formation was observed to occur for the more soluble phenols, indicating that peroxidase-mediated immobilization can be more effective for the containment of phenols that are highly mobile in soil and groundwater.

CONCLUSIONS

This research illustrates the potential of peroxidase-mediated oxidative coupling processes to immobilize phenolic contaminants in soil and aquifer environments. The phenolic oligomers produced during peroxidase-mediated polymerization can bind strongly to the soil organic matter and mineral surfaces. Addition of enzyme and cofactors at field sites contaminated with phenols may be accomplished through injection wells or by constructing permeable reactive barriers with immobilized enzymes. Enzymes immobilized on clays and soils can retain their ability to mediate oxidative coupling processes over long periods (*42*). Contact with the enzyme (and cofactor) would result in polymerization of phenols and their subsequent binding to soil or aquifer solids. Such polymerization and binding of phenols can produce large reductions in contaminant bioavailability (*30*, *33*, *34*) arising from the low aqueous solubility of the polymeric products. Chemical alterations such as the fortuitous dechlorination of chlorophenols during polymerization may also lead to lowered toxicity (*31*, *32*). However, it is necessary to investigate adequate means of delivering the enzyme and cofactors to the affected areas at contaminated sites. Future research work should also more closely represent "real-world" scenarios by studying the effectiveness of peroxidase-mediated immobilization in the presence of disparate contaminants.

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LITERATURE CITED

(1) Clean Water Act. Section 307. In *Environmental Statutes*; Rockville, MD, 1985.
(2) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*; Wiely: New York, 1993.
(3) Isaacson, P. J.; Frink, C. R. Nonreversible sorption of phenolic compounds by sediment fractions: the role of sediment organic matter. *Environ. Sci. Technol*. **1984**, *18*, 43−48.
(4) Robinson, K. G.; Novak, J. T. Fate of 2,4,6-trichlorophenol bound to dissolved humic acid. *Water Res.* **1994**, *28*, 445−452.
(5) Xing, B.; McGill, W. B.; Dudas, M. J.; Maham, Y.; Helper, L. Sorption of phenol by selected biopolymers: isotherms, energetics, and polarity. *Environ. Sci. Technol.* **1994**, *28*, 466−473.
(6) Benoit, P.; Barriuso, E.; Houot, S.; Calvet R. Influence of the nature of soil organic matter on the sorption−desorption properties of 4-chlorophenol, 2,4-dichlorophenol and the herbicide 2,4-D. *Eur. J. Soil Sci.* **1996**, *47*, 567−578.
(7) Divincenzo, J. P.; Sparks, D. L. Slow sorption kinetics of pentachlorophenol on soil. *Environ. Sci. Technol*. **1997**, *31*, 977−983.
(8) Sabbah, I.; Rebhun, M. Adsorption−desorption of trichlorophenol in water−soil systems. *Water Environ Res.* **1997**, *69*, 1032−1038.
(9) Yost, E. C.; Anderson, M. A. Absence of phenol adsorption on goethite. *Environ. Sci. Technol*. **1984**, *18*, 101−106.
(10) Chiou, C. T.; Porter, P. E.; Schmedding, D. W. Partition equilibria of nonionic organic compounds between soil organic matter and water. *Environ. Sci. Technol*. **1983**, *17*, 227−231.
(11) Gschwend, P. M.; Wu, S. On the constancy of sediment−water partition coefficients of hydrophobic organic pollutants. *Environ. Sci. Technol*. **1985**, *19*, 90−96.
(12) Lee, L. S.; Rao, P. S. C.; Nkedi-Kkizza, P.; Delfino, J. J. Influence of solvent and sorbent characteristics on distribution of pentachlorophenol in octanol−water and soil−water systems. *Environ. Sci. Technol*. **1990**, *24*, 654−661.
(13) Weber, W. J., Jr.; LeBoeuf, E. J.; Young, T. M.; Huang, W. Contaminant interactions with geosorbent organic matter: insights drawn from polymer sciences. *Water Res.* **2001**, *35*, 853−868.
(14) Gundersen, J. L.; Macintyre, W. G.; Hayle, R. C. pH-dependent sorption of chlorinated guaiacols on estuarine sediments: the effect of humic acids and TOC. *Environ. Sci. Technol.* **1997**, *31*, 188−193.
(15) Khodadoust, A. P.; Suidan, M. T.; Sorial, G. A.; Dionysiou, D. D.; Brenner, R. C. Desorption of pentachlorophenol from soils using mixed solvents. *Environ. Sci. Technol*. **1999**, *33*, 4483−4491.
(16) Bhandari, A.; Novak, J. T.; Berry, D. F. Binding of 4-monochlorophenol to soil. *Environ. Sci. Technol*. **1996**, *30*, 2305−2311.
(17) Bhandari, A.; Novak, J. T.; Burgos, W. D.; Berry, D. F. Irreversible binding of chlorophenols to soil and its impact on bioavailability. *J. Environ. Eng.* **1997**, *123*, 506−513.
(18) Bollag, J.-M. Decontaminating soil with enzymes. *Environ. Sci. Technol.* **1992**, *26*, 1876−1881.
(19) Stone, A. T. Reductive dissolution of manganese(III/IV) oxides by substituted phenols. *Environ. Sci. Technol.* **1987**, *21*, 979−988.
(20) McBride, M. B. Adsorption and oxidation of phenolic compounds by iron and manganese oxides. *Sci. Soc. Am. J.* **1987**, *51*, 1466−1472.
(21) Larson, R. A.; Weber, E. J. In *Reaction Mechanism in Environmental Organic Chemistry*; Lewis Publishers: Chelsea, MI, 1994.
(22) Bollag, J.-M.; Liu, S.-Y.; Minard, R. D. Cross-coupling of phenolic humus constitutents and 2,4-dichlorophenol. *Soil Sci. Soc. Am. J.* **1980**, *44*, 52−56.
(23) Suflita, J. M.; Bollag, J.-M. Polymerization of phenolic compounds by a soil−enzyme complex. *Soil Sci. Soc. Am. J.* **1981**, *45*, 297−302.
(24) Berry, D. F.; Boyd, S. A. Oxidative coupling of phenols and anilines by peroxidase: Structure−activity relationships. *Soil Sci. Am. J.* **1984**, *48*, 565−569.
(25) Liu, S.-Y.; Minard, R. D.; Bollag, J.-M. Soil-catalyzed complexation of the pollutant 2,6-diethylaniline with syringic acid. *J. Environ. Qual.* **1987**, *16*, 48−53.
(26) Sarkar, J. M.; Malcolm, R. L.; Bollag, J.-M. Enzymatic coupling of 2,4-dichlorophenol to stream fulvic acid in the presence of oxidoreductases. *Soil Sci. Soc. Am. J.* **1988**, *52*, 688−694.
(27) Tatsumi, K.; Freyer, A.; Minard, R. D.; Bollag, J.-M. Enzyme-mediated coupling of 3,4-dichloroaniline and ferulic acid: a model for pollutant binding to humic materials. *Environ. Sci. Technol.* **1994**, *28*, 210−215.

(28) Bollag, J.-M.; Shuttleworth, K. L.; Anderson, D. H. Laccase-mediated detoxification of phenolic compounds. *Appl. Environ. Microbiol*. **1988**, *54*, 3086−3091.
(29) Ruggiero, P.; Sarkar, J. M.; Bollag, J.-M. Detoxification of 2,4-dichlorophenol by a laccase immobilized on soil or clay. *Soil Sci.* **1989**, *147*, 361−370.
(30) Dec, J.; Bollag, J.-M. Detoxification of substituted phenols by oxidoreductive enzymes through polymerization reactions. *Arch. Environ. Contam. Toxicol.* **1990**, *19*, 543−550.
(31) Dec, J.; Bollag, J.-M. Dehalogenation of chlorinated phenols during oxidative coupling. *Environ. Sci. Technol.* **1994**, *28*, 484−490.
(32) Dec, J.; Bollag J.-M. Effect of various factors on dehalogenation of chlorinated phenols and anilines during oxidative coupling. *Environ. Sci. Technol.* **1995**, *29*, 657−663.
(33) Dec, J.; Bollag, J.-M. Microbial release and degradation of catechol and chlorophenols bound to synthetic humic acid. *Soil Sci. Soc. Am. J.* **1988**, *52*, 1366−1371.
(34) Dec, J.; Shuttleworth, K. L.; Bollag, J.-M. Microbial release of 2,4-dichlorophenol bound to humic acid or incorporated during humification. *J. Environ. Qual.* **1990**, *19*, 546−551.
(35) Berry, D. F.; Boyd, S. A. Decontamination of soil through enhanced formation of bound residues. *Environ. Sci. Technol*. **1985**, *19*, 1132−1133.
(36) Bhandari, A.; Xu F. Impact of peroxidase addition on the sorption−desorption behavior of phenolic contaminants in surface soils. *Environ. Sci. Technol.* **2001**, *35*, 3163−3168.
(37) Oritz de Montellano, P. R.; David, S. K.; Ator, M. A.; Tew, D. Mechanism-based inactivation of horseradish peroxidase by sodium azide. Formation of *meso*-azidoprotoporphyrin IX. *Biochemistry* **1988**, *27*, 5470−5476.
(38) Xu, F. Horseradish peroxidase mediated sorption and binding of phenol, *o*-cresol, 2,4-dichlorophenol and 1-naphthol to surface soils. Ph.D. dissertation, Kansas State University, 2002.
(39) Ball, W. P.; Roberts, P. V. Long-term sorption of halogenated organic chemicals by aquifer material, 2. Intraparticle diffusion. *Environ. Sci. Technol*. **1991**, *25*, 1237−1249.
(40) Buchanan, I. D.; Nicell, J. A. Model development for horseradish peroxidase catalyzed removal of aqueous phenol. *Biotechnol. Bioeng.* **1997**, *54*, 251−261.
(41) Martin, J. P.; Haider, K. A comparison of the use of phenolase and peroxidase for the synthesis of model humic acid-type polymers. *Sci. Soc. Am. J.* **1980**, *44*, 983−988.
(42) Sarkar, J. M.; Leonowicz, A.; Bollag, J.-M. Immobilization of enzymes on clays and soils. *Soil Biol. Biochem*. **1989**, *21*, 223−230.

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