EFFECT OF PEROXIDASE ADDITION ON SORPTION, DESORPTION, AND BINDING OF PHENOLIC MIXTURES IN SOIL

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ABSTRACT

Peroxidase enzymes can enhance the rate and extent of contaminant binding to soil organic matter and immobilize the contaminant within the soil matrix, and thus prevent its transport into the subsurface. This study focused on evaluating the binding of phenol, dichlorophenol, and naphthol, individually and as mixtures, on two sandy loams belonging to the Haynie series. U-ring-^{14}C-labeled target chemicals were used as radio tracers to track the distribution of the pollutant among various soil components. Bottle-point adsorption experiments with constant soil dosage were conducted for a period of seven days followed by sequential desorption of the "quickly desorbing" fraction with synthetic groundwater. The "slowly desorbing" chemicals were removed by multiple extractions with methanol. The chemicals associated with humic/fulvic acids and the soil (humin) fraction were determined by combustion of soil before and after alkali-extraction. Results illustrated that enzyme addition increased adsorption of phenol, and DCP and lead much more than desorption hysteresis, whether they were present alone or as a mixture. Addition of enzyme also resulted in dramatic enhancement in contaminant binding. The presence of co-contaminant did not affect adsorption or desorption of phenol or DCP, but to some extent decreased binding to HA/FA and soil humin fraction. Addition of enzyme did not enhance adsorption of naphthol but increased binding to HA/FA and soil humin fraction.

Key words: *adsorption, binding, phenolic mixtures, horseradish peroxidase*

INTRODUCTION

Substituted phenols are classified as priority pollutants because of their multiple toxic health effects at very low concentrations (Clean Water Act, 1985). These chemicals constitute an important class of organic contaminants commonly associated with polluted soils and sediments. They may enter the environment during uncontrolled discharges or accidental spills, or accumulate as intermediates during the incomplete biodegradation of gasoline constituents or pesticide mixtures (Guerin and Jones, 1988; Heitkamp and Cerniglia, 1988). Moreover, several of these chemicals and their precursors, including fuel hydrocarbons and pesticides, are commonly found to coexist at contaminated sites (Riley et al., 1992; NRC, 1994; USAF, 1996). Soil and sediments polluted with such chemical mixtures are a major cause of concern because of the high risks posed to ecosystem health and the difficulty of treatment using conventional remediation approaches. A compelling need, therefore, exists to evaluate alternative remediation strategies that can effectively and economically lower the risks of environmental exposure from these compounds by chemically altering the contaminants in ways that significantly reduce their interaction with target receptors.

Engineering remediation schemes to influence the fate and transport of organic contaminants in soils and sediments requires a thorough understanding of the governing physical, chemical, and biological processes in complex natural environments. Sorption processes between organic chemicals and soil components are well documented (Chiou et al., 1983; Pignatello, 1989; Ball and Roberts, 1991; Robinson and Novak, 1994; Weber and

Huang, 1996). Chemical reactions between these pollutants and soil/sediment matrices have, however, received only limited attention. There is increasing evidence that chemical interactions between organic pollutants and soil components, specifically reactions catalyzed by transition metal oxides and soil enzymes, can significantly affect the fate of contaminants in soils and the subsurface, and potentially alter the associated health risks from the chemicals (Wang et al., 1986; Voudrais and Reinhard, 1986; Nannipieri and Bollag, 1991; Bollag, 1992; Gianfreda and Bollag, 1994).

Interactions of Organic Contaminants with Soil Organic Matter

Soil organic matter (SOM) has been implicated as one of the fundamental factors controlling the fate of organic contaminants in soils and sediments (Chiou et al., 1983; Gschwend and Wu, 1985; Lee et al., 1990; Rutherford et al., 1992). Sorption capacities of soils for organic contaminants are strongly influenced by the content and chemical composition of SOM (Karickhoff et al., 1979; Garbarini and Lion, 1986; Gauthier et al., 1987; Gratwohl, 1990; Rutherford et al., 1992; Weber and Huang, 1996). In the case of ionogenic organic contaminants that include phenols and anilines, properties of the soil solution such as pH and ionic strength, as well as the acid dissociation constant (pK_a) of the chemical, can affect the pollutant's sorption behavior (Schellenberg et al., 1984; Westall et al., 1985; Lee et al., 1991). Desorption of organic contaminants from soils has been observed to involve a relatively fast initial release of the sorbate followed by a prolonged and increasingly slower desorption, suggesting the possibility that a fraction of the solute may remain bound or sequestered within the soil matrix (Schwarzenbach and Westall, 1981; DiToro and Horzempa, 1982; Steinberg et al., 1987; Pavlothasis and Mathavan, 1992; Xing et al., 1996; Cornelissen et al., 1997). This desorption resistant fraction has been found to increase with increasing solute-sorbent contact time (Steinberg et al., 1987).

Soils contain large concentrations of extracellular enzymes that can catalyze degradation and biosynthesis reactions in the soil environment. These organic catalysts are often protected against natural degradation by their attachment to soil organic and mineral domains. Several soil enzymes, including peroxidases, laccases, and polyphenol oxidases, are capable of catalyzing chemical reactions that result in the polymerization of phenolic chemicals via reactions analogous to those catalyzed by transition metal oxides. Sjoblad et al. (1976) were among the first to demonstrate the ability of a soil fungus-derived phenoloxidase to polymerize phenols. Suflita and Bollag (1980) later studied the polymerization of methoxy-phenol and chloronaphthol by a soil-enzyme complex. Klibanov and coworkers utilized horseradish-derived peroxidase to investigate the polymerization of aromatic amines and phenols in aqueous systems (Klibanov and Morris, 1981; Klibanov et al., 1983).

Hydroxylated aromatic compounds have been shown to form covalently linkages with model soil organic matter as a result of enzyme-

catalyzed oxidative coupling. Bollag et al. (1980) observed the cross coupling of 2,4-dichlorophenol (DCP) to model humus constituents that included orcinol, syringic acid, vanillic acid, and vanillin. Bollag and Liu (1985) observed similar laccase-catalyzed copolymerization of syringic acid with other halogenated phenols including 4-chlorophenol, 2,6-DCP, 4-bromo-2-chlorophenol, 2,3,6-, and 2,4,5-trichlorophenol, 2,3,5,6-tetrachlorophenol and pentachlorophenol. Oxidative coupling catalyzed by three oxidoreductases—tyrosinase, peroxidase, and laccase—was demonstrated for 2,4-DCP and a stream fulvic acid (Sarkar et al., 1988). This study also illustrated that enzyme-catalyzed incorporation of the target chemical occurred over a wide range of pH and temperature. Recent work by the PI and co-investigators has attributed irreversible binding of aromatic compounds observed during sorption-desorption experiments to enzyme and transition metal oxide-catalyzed oxidative coupling of the contaminant to SOM (Bhandari et al., 1996; 1997; 1998; Burgos et al., 1999). Other researchers have investigated incorporation of pesticides such as atrazine into soils and have attributed a significant portion of the binding to oxidative polymerization reactions (Barriuso and Koskinen, 1996). Recently, our laboratory reported a ten-fold increase in the amount of phenol and *o*-cresol associated with soil in the presence of peroxidase (Bhandari and Cho, 1999). The enhanced sorption was attributed to the greater hydrophobicity of the polymerized products and possible covalent bond formation between the phenols and SOM. This paper describes the effect of peroxidase addition to soils containing a mixture of phenolic contaminants.

METHODS AND MATERIALS

Materials

U-^{14}C-phenol, U-^{14}C-2,4-dichlorophenol (DCP), and carbon-1-^{14}C labeled 1-naphthol were purchased from Sigma Chemicals with specific activities of 14.3, 20.9, and 9.6 mCi/mmol, respectively. These were dissolved in methanol to prepare stock solutions and used without further purification. Unlabeled chemical stock solutions were also prepared in methanol. All labeled and non-labeled stock solutions were stored at sub-zero temperatures. Horseradish peroxidase (Type II, RZ:2.2, 240 purpurogallin units/ mg) and hydrogen peroxide (30% w/w, 8.82 mol/L) were purchased from Sigma Chemicals and used without further purification.

Soil

Two soils were collected from an agricultural field and an adjacent forested site near the city of Manhattan, Kansas. These soils belong to the Haynie series (fine sandy loam). The soils were collected aseptically and transported to the laboratory in coolers. Soils were sieved to pass through 1-mm and 500-µm sieves. Each soil was split into smaller representative fractions using the coning and quartering technique. The soil fractions were sterilized using a multiple autoclaving procedure that included subjecting the soils to sequential autoclaving and incubation to neutralize all spore-forming bacteria. The soils were incubated twice for 48 hours and

autoclaved three times for 50 minutes over a five-day period.

When mixed with water, both soils released natural organic matter into solution. To avoid working with a three-phase system (soil, water, and dissolved organic material), the soils were washed several times with warm synthetic groundwater. Field soil was washed seven times and forest soil was washed 11 times. After washing, the field soil contained 44% sand, 42% silt and 14% clay; OM content was 1.7% and CEC (cation exchange capacity) was 9.7 meq/100 g. The forest soil contained 56% sand, 38% silt, and 6 % clay; OM content was 2.6%; CEC was 10.9 meq/100g. Synthetic groundwater (GW) was prepared by adding 500 mg/L of the biocide sodium azide into a pH 7 phosphate buffer solution. The phosphate buffer contained 1.8 mM KH_2PO_4, and 2.82 mM K_2HPO_4 with an ionic strength of about 18 mM. The washed soils were dried at 35 ^oC and homogenized with a mortar and pestle. The SOM contents of the washed soils were 1.7% and 2.6% for the field and forest soils, respectively. All prepared soils were stored at sub-zero temperatures in glass bottles.

Adsorption and Desorption Experiments

Three initial aqueous concentrations (C_0) of 5, 50, 500 µM were used in adsorption experiments. All solutions were prepared in synthetic groundwater. Known volumes and activities of the radioactive target chemicals were added to corresponding non-radioactive target chemical solutions. For the chemical mixture studies, parallel experiments were conducted with one target chemical being labeled while the other chemical was non-labeled. Three sets of completely mixed-batch reactors (CMBRs) (triplicate) were used for each concentration of the test compound.

Each CMBR was prepared with 5.0 grams of soil (dry wt) and completely filled with a solution of labeled and non-labeled chemical(s) (approximately 13 mL) to minimize any headspace in the reactor. Enzyme (horseradish peroxidase) and H_2O_2 were added into the reactors with the enzyme concentration of two Sigma activity units (AU) of peroxidase per mL of solution and sufficient H_2O_2 to achieve a solution concentration equal to that of the target chemicals. The CMBRs were immediately sealed with Teflon-lined phenolic caps to minimize the volatilization of chemicals. Non-HRP experiments were conducted identically with no enzyme or H_2O_2 addition. Control experiments to quantify chemical losses due to the volatilization or adsorption to reactor components were conducted at the same time with no soil or enzyme+H_2O_2 addition.

After the tubes were capped, the contents were mixed with a touch-mixer. The reactors were then placed in a tumbler and allowed to mix for seven days at room temperature. At the end of seven days, the tubes were centrifuged at 2200 *g* for 45 minutes and a 250 µL aliquot of the supernatant was removed from each tube and transferred into a scintillation vial with 5 mL scintillation cocktail (ScintSafe Plus 50%, Fisher Scientific). The samples were allowed to stand overnight to minimize chemiluminescence, after which the radioactivity was enumerated as disintegrations per minute (dpm) using a

Beckman 6500 liquid scintillation counter (LSC) with quench and luminescence corrections.

After the 250 µL aliquot was removed from the tubes at the end of the adsorption experiment, the remaining supernatant was pipetted out and replaced with clean synthetic GW. The tubes were capped tightly, mixed with a touch-mixer, and placed in the tumbler for overnight reequilibration (desorption). After 24 h the CMBRs were centrifuged and the supernatants drawn for analysis on the LSC. Sequential desorption experiments were repeated until the radioactivity in the supernatant was reduced to less than 50 dpm (below detection limit).

Extraction and Combustion

Methanol extraction was conducted following desorption with synthetic GW to remove any strongly adsorbed chemicals. Extractions were conducted in the same way as GW desorption except the fresh solution was methanol, instead of GW solution. Solvent extractions were repeated until the radioactivity in the supernatant was reduced to below detection limit. The net methanol-extractable fraction (slowly desorbing fraction) was determined by mass balance.

The solvent-extracted soils were dried in a fume hood and one half of the soil in each tube was directly combusted at 925°C in an OX-500 Biological Material Oxidizer (BMO, R.J. Harvey and Associates). The $^{14}CO_2$ produced during combustion was trapped in Harvey Carbon-14 Cocktail and analyzed by the LSC to determine the amount of target chemicals bound to soil and humic substances The second half of the soil was extracted with alkali (0.1 N NaOH in a N_2 atmosphere) several times to remove humic and fulvic acids from soil. The soil containing the non-alkali extractable SOM (humin fraction) was combusted in the oxidizer and the chemicals associated with this fraction quantified by the radioactivity liberated as $^{14}CO_2$. The fraction of chemicals associated with alkali-extractable humic acid and fulvic acid was determined by mass balance. Figure 1 illustrates the complete experimental process.

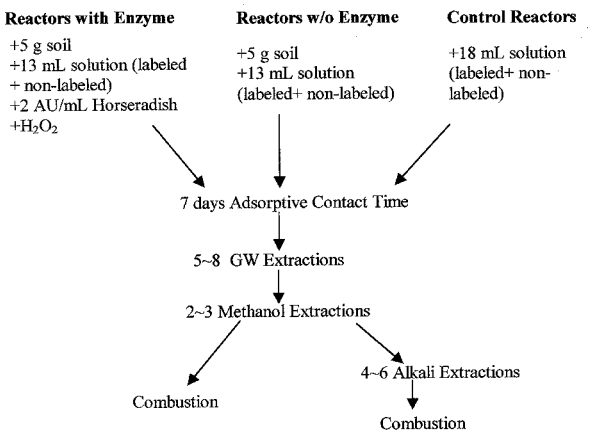

Figure 1. Schematic of experimental procedures.

The adsorption data were fitted to the Freundlich model described as

$$q_e = K_F C_e^n or \log q_e = n \log C_e + \log K_F$$

where q_e and C_e represent the solid- and aqueous-phase concentrations of the target chemical, respectively. K_F is referred to as the Freundlich constant, which is a measure of the sorption capacity of the sorbent. The Freundlich *n* measures sorption linearity which is related to the heterogeneity of sorption sites on the sorbent.

Sorption-desorption behavior of organic contaminants in soils is often characterized by the occurrence of hysteresis. Possible reasons for this behavior include the presence of "ink-

bottle" type pores that can trap the sorbate or, more likely, the occurrence of irreversible changes on the sorbent surface, resulting in a desorption process that is actually different from the adsorption process (Adamson, 1990). In this study, we measured hysteresis using the Hysteresis Index (H.I.) defined by Huang and Weber (1997) as

$$HI = \frac{q_e^d - q_e^a}{q_e^a} | T, C_e |$$

where q_e^a and q_e^d are solid-phase solute concentrations for the adsorption and desorption experiments, respectively; and T and C_e specify conditions of constant temperature and residual aqueous phase concentration. An H.I. of zero is indicative of no hysteresis. H.I. values in the studies discussed here were calculated at C_e values of 10 µM.

RESULTS AND DISCUSSION

Several terms need to be defined for the following discussion. The water-extractable component of the adsorbed chemical was operationally defined as the "quickly desorbing" fraction. The fraction of the adsorbed chemical that was removed by solvent extraction was defined as the "slowly desorbing"component. The solute associated with the humic/ fulvic acid fraction represents the contaminant bound to these organic macromolecules, as well as solute trapped within the microcrystalline, glassy domains of the humic and fulvic acids. The solute resistant to water, solvent, and alkali extractions represents the contaminant that is actually bound to the humin component of the SOM, as well as solute trapped in the micropores associated with the mineral domain.

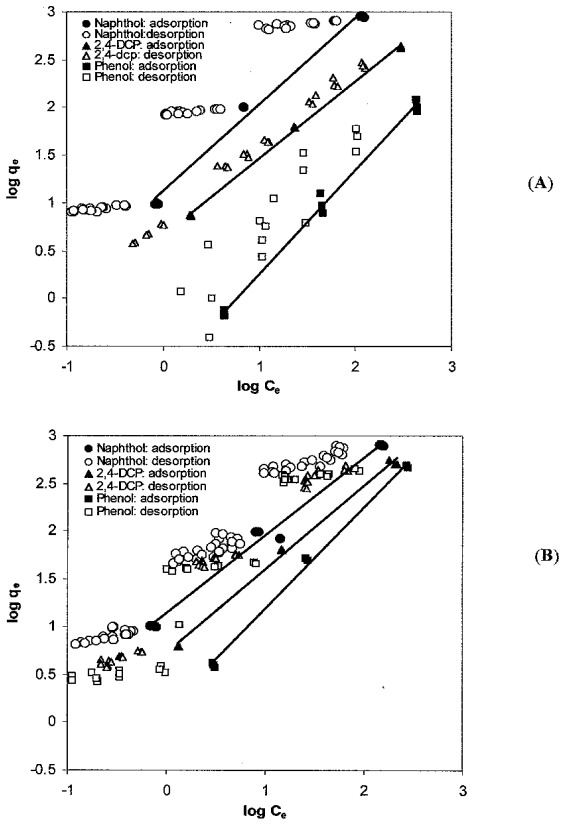

Figure 2. Adsorption-desorption behavior of phenol, 2,4-DCP, and naphthol (A) in the abscences of HRP, (b) in the presence of HRP (field soil).

Sorption Isotherms of Single-Target Chemicals

The effect of peroxidase addition on sorption-desorption of phenol, DCP, and naphthol on field and forest soils was investigated by comparing adsorption and desorption behaviors of target chemicals with and without enzyme addition. Figure 2 illustrates adsorption-desorption data of phenol, DCP, and naphthol in the presence and absence of enzyme for field soil. The corresponding data for the forest soil are summarized in Table 1. Figure 2A describes adsorption-desorption of phenol, DCP, and naphthol when no HRP was added. Figure 2B is the data upon HRP addition.

Adsorption of phenol was dramatically enhanced by addition of enzyme. The Freundlich *n* value for phenol in presence of enzyme, 1.06, was almost the same as that in the absence of enzyme, 1.09. But logK_F value increased from –0.84 to 0.13. The adsorption of DCP was increased by the addition of HRP, although the increment was not so obvious as that of phenol. The Freundlich *n* value increased from 0.81 to 0.88 and log K_F from 0.66 to 0.72 upon HRP addition. The adsorption of naphthol didn't show significant increase upon HRP addition.

From the adsorption data of phenol, DCP, and naphthol, it can be noted that the effect of enzyme addition can be correlated with the hydrophobicities of target chemicals. The addition of enzyme resulted in a large enhancement in adsorption of phenol to soil. The addition of enzyme increased adsorption of DCP, although to a smaller extent than phenol. Enzyme addition, however, had no effect on adsorption of naphthol. Enzymatic polymerization was efficient when the substrate was available in solution. Increases in hydrophobicity resulted in decrease in enzyme-catalyzed adsorption as illustrated by naphthol.

The effect of enzyme addition on desorption of DCP, naphthol, and phenol is also shown

Table 1, part 1. Freundlich isotherm parameters (*n* and log K_F) and Hysteresis Index (H.I.) values for the different soil-contamininat-treatment combinations studied.

Soil	Target-Chemical	HRP[1]	*n*	log K_F	H.I.[2]
Field	Phenol	No	1.09	-0.84	0.96
		Yes	1.06	0.13	19.4
	DCP	No	0.81	0.66	0.07
		Yes	0.88	0.72	5.31
	Naphthol	No	0.91	1.12	5.61
		Yes	0.82	1.13	3.47
Field	Phenol in DCP/Phenol Mixture	No	0.88	-0.56	0.78
		Yes	0.98	0.28	12.8
	Phenol in Phenol/Naphthol Mixture	No	0.91	0.63	0
		Yes	1.01	0.038	16.5
	DCP in DCP/Phenol Mixture	No	0.74	0.56	0
		Yes	0.91	0.66	5.76
	DCP in DCP/Naphthol Mixture	No	0.79	0.52	0
		Yes	1.11	0.49	10.76

[1]HRP = horseradish peroxidase enzyme,

[2] H.I. values were determined using q_e^a and q_e^d values at C_e = 10μM

in Figure 2. Addition of enzyme drastically reduced desorption of phenol and DCP. There was no obvious hysteresis in the desorption of phenol or DCP when no enzyme was added. The addition of enzyme, however, resulted in obvious hysteresis in desorption of phenol or DCP, and increased the H.I. value from 0.96 to 19.4 for phenol and from 0.07 to 5.31 for DCP. Very small amounts of the adsorbed target chemicals were desorbed upon the addition of enzyme. An interesting result was observed in the desorption of naphthol when no enzyme was added; the major part of adsorbed naphthol resisted water extraction while a large amount of adsorbed phenol and DCP was extracted with water. It appears that naphthol was adsorbed more tightly than phenol and DCP. Burgos et al. (1996) showed similar resistance to naphthol desorption in the absence of enzyme. Similar results were observed in forest soil (Table 1).

Sorption Isotherms of Phenolic Mixtures

Since usually more than one phenolic chemical is present in contaminated sites, the effect of the presence of phenolic co-contaminants on the adsorption-desorption was investigated with and without addition of peroxidase. In these studies with mixtures consisting of two

Table 1, part 2. Freundlich isotherm parameters (n and log K_F) and Hysteresis Index (H.I.) values for the different soil-contamininat-treatment combinations studied.

Soil	Target Chemical	HRP[1]	n	log K^F	H.I.[2]
Forest	Phenol	No	1.02	-0.65	4.02
		Yes	1.03	-0.0022	20.9
	DCP	No	0.91	0.77	0
		Yes	0.89	0.65	6.07
	Naphthol	No	1.0	1.0	0
		Yes	0.80	0.80	9.37
Forest	Phenol in DCP/Phenol Mixture	No	0.91	-0.53	0
		Yes	0.92	0.093	15.9
	Phenol in DCP/Naphthol Mixture	No	0.93	-0.60	0
		Yes	1.09	-0.37	24.6
	DCP in DCP/Phenol Mixture	No	0.87	0.56	0
		Yes	0.82	0.76	3.37
	DCP in DCP/Naphthol Mixture	No	0.89	0.65	0
		Yes	0.88	0.68	6.59

[1]HRP = horseradish peroxidase enzyme,

[2] H.I. values were determined using q_e^a and q_e^d values at $C_e = 10\mu M$

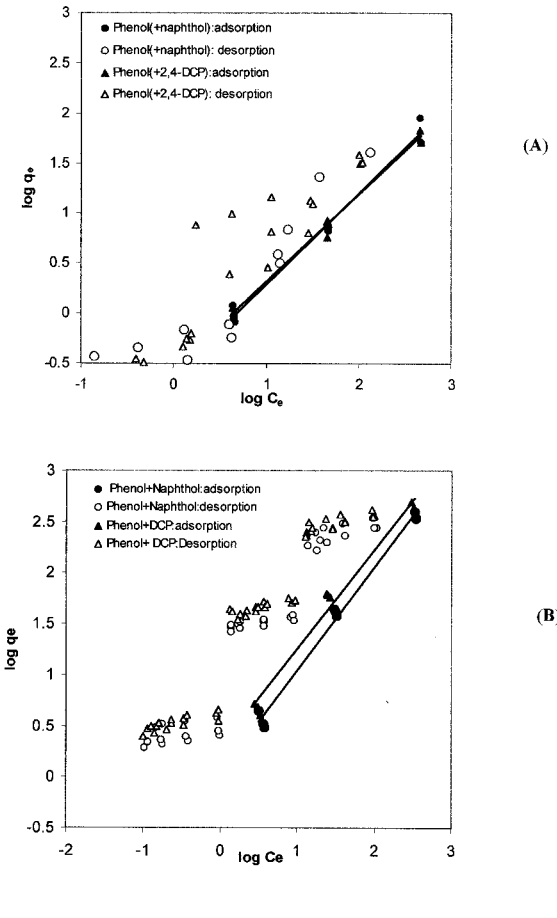

Figure 3. Adsorption-desorption behavior of phenol present with other phenolic chemicals (A) in the abscence of HRP, (B) in the presence of HRP (field soil).

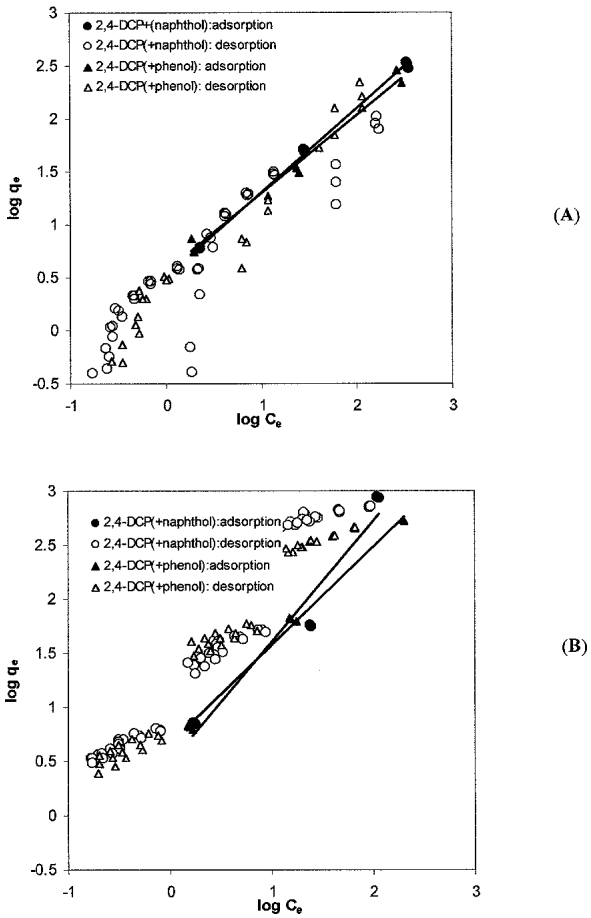

Figure 4. Adsorption-desorption behavior of 2,4-DCP present with other phenolic chemicals (A) in the abscence of HRP enzyme, (B) in the presence of HRP enzyme (field soil).

chemicals, the initial concentration of each chemical was 5 µM, 50 µM, and 500µM, respectively. In Figures 3 and 4, the chemical in the parenthesis is the unlabeled one. Figure 3 is the data of adsorption-desorption of phenol in the presence of DCP and naphthol in field soil. Freundlich isotherm fits were used to model the adsorption behavior. Figure 4 is the data of adsorption-desorption of DCP in the presence of phenol and naphthol in field soil. The data corresponding to the forest soil are summarized in Table 1.

Comparing the adsorption-desorption data of phenol alone and phenol in presence of DCP, or naphthol, there appears to be no obvious difference between the adsorption of phenol alone and phenol in the presence of co-contaminant, especially under low-solution concentration. Under higher solution concentration, adsorption of phenol was reduced a little bit in the presence of DCP or naphthol, which may indicate the competition of limited adsorption sites under higher solute concentration. The data of adsorption and desorption of DCP alone, in

the presence of phenol or naphthol (Figure 4), also confirms that under low-solute concentrations, there are no obvious differences in adsorption whether DCP is present alone, or in the presence of phenol or naphthol.

Figures 3B and 4B are the data of adsorption, desorption of phenol, and DCP, respectively, in the presence of co-contaminant upon addition of peroxidase. It can be seen that there is no obvious difference between the adsorption-desorption of phenol present alone, or in the presence of DCP or naphthol. Similar results were observed in the case of DCP. These results clearly demonstrate that not only is the adsorption of a single chemical (phenol or DCP) greatly enhanced upon enzyme addition, but adsorption of these chemicals in the presence of co-contaminants is also greatly increased. In the presence of a second phenolic chemical, the adsorption of phenol or DCP did not show obvious differences from when the target chemical was present alone, irrespective of which phenolic chemical was present. The addition of HRP dramatically enhanced the adsorption of phenol or DCP to the field soil in the presence of co-contaminant.

Addition of HRP also affected the desorption of phenolic mixtures. Desorption of target chemicals in all mixtures was reduced dramatically compared to the case when no enzyme was added. H.I. values (Table 1) of phenol in the presence of DCP increased from 0.78 to 12.8 upon the addition of HRP, from 0 (almost desorbed completely) to 16.5 in the presence of naphthol. Similar results were observed for H.I. values of DCP in the presence of phenol or naphthol. Similar results were observed in the case of forest soil (Table 1).

Distribution of Target Chemicals in Soil Fractions

Figure 5A represents the distribution of DCP in different soil fractions in the presence/absence of enzyme. Figures 5B and 5C represent the data for DCP in the presence of naphthol and phenol, respectively. In the absence of enzyme at an initial solute concentration of 50 µM, more than 30% of DCP adsorbed was removed by water extraction, whether DCP was present alone or with co-contaminant. Under higher initial solute concentration, a greater fraction of DCP adsorbed was removed by water extraction. The presence of co-contaminant increased the percentage of the quickly desorbing fraction. Less than 5% of DCP adsorbed was bound to humic/fulvic acids and soil fraction if no enzyme was added. Almost 50% or even more of adsorbed DCP was removed by methanol extraction (slowly desorbing fraction).

The addition of enzyme dramatically altered the percentage distribution of DCP associated with different soil fractions. In the absence of co-contaminants, 55% of DCP adsorbed was bound to humic /fulvic acids and 7% was bound to soil humin fraction upon addition of enzyme. The presence of co-contaminant decreased the fraction of DCP bound to humic/fulvic and the soil humin fraction to some degree. But still more than 25% of DCP adsorbed was associated with these fractions. Similar observations were found in experiments with initial solute concentrations of

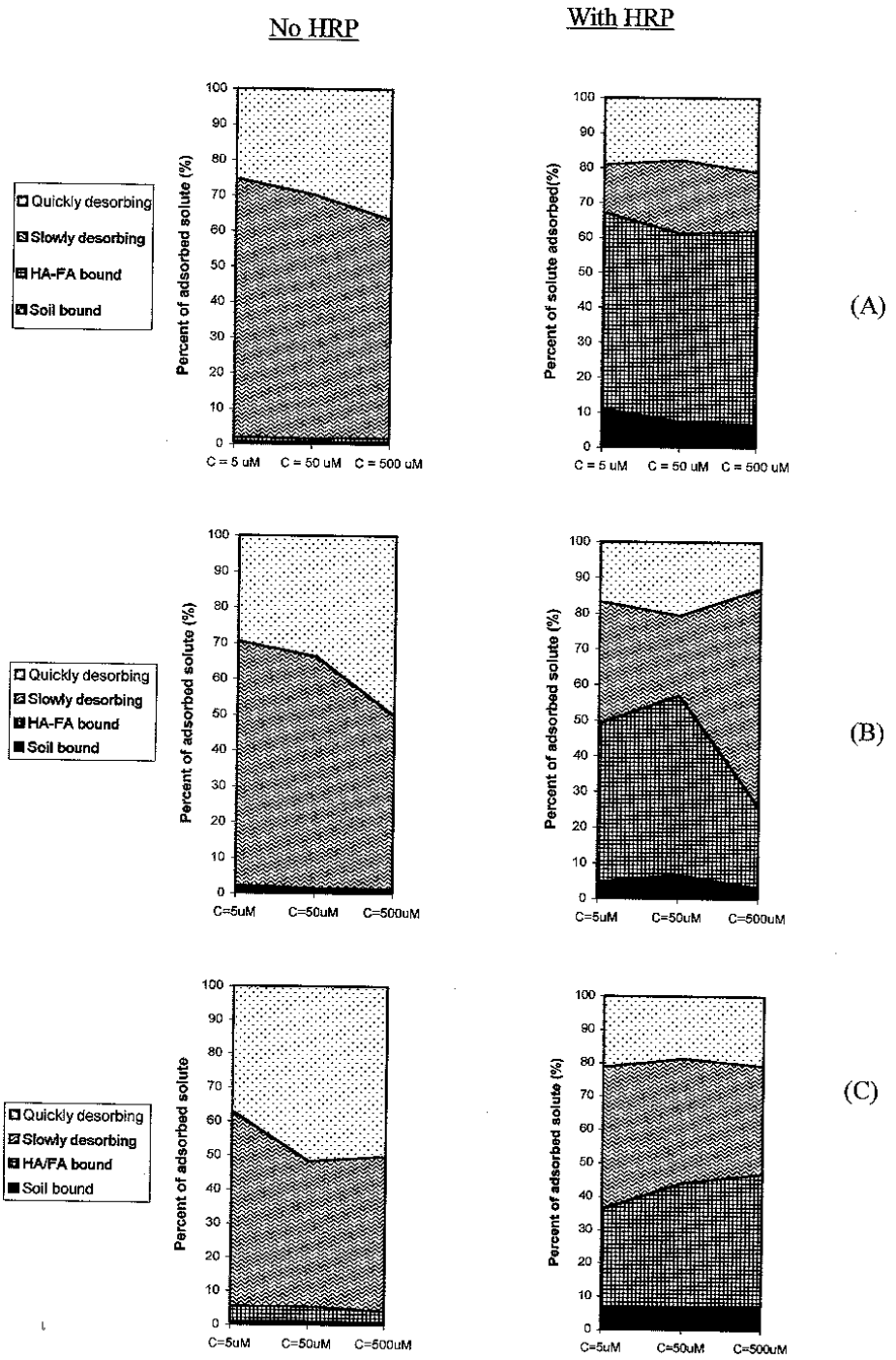

Figure 5. Distribution of 2,4-DCP in field soil, in the abscence/presence of HRP enzyme, (A) present alone, (B) present with naphthol, (C) present with phenol.

5 µM and 500 µM. The presence of naphthol decreased more than the fraction of DCP in humic/fulvic acids and soil fraction. That could have resulted from the competition of binding sites between chemicals, which may have decreased the availability of DCP. Naphthol showed higher competition with DCP than phenol since naphthol is more hydrophobic than phenol.

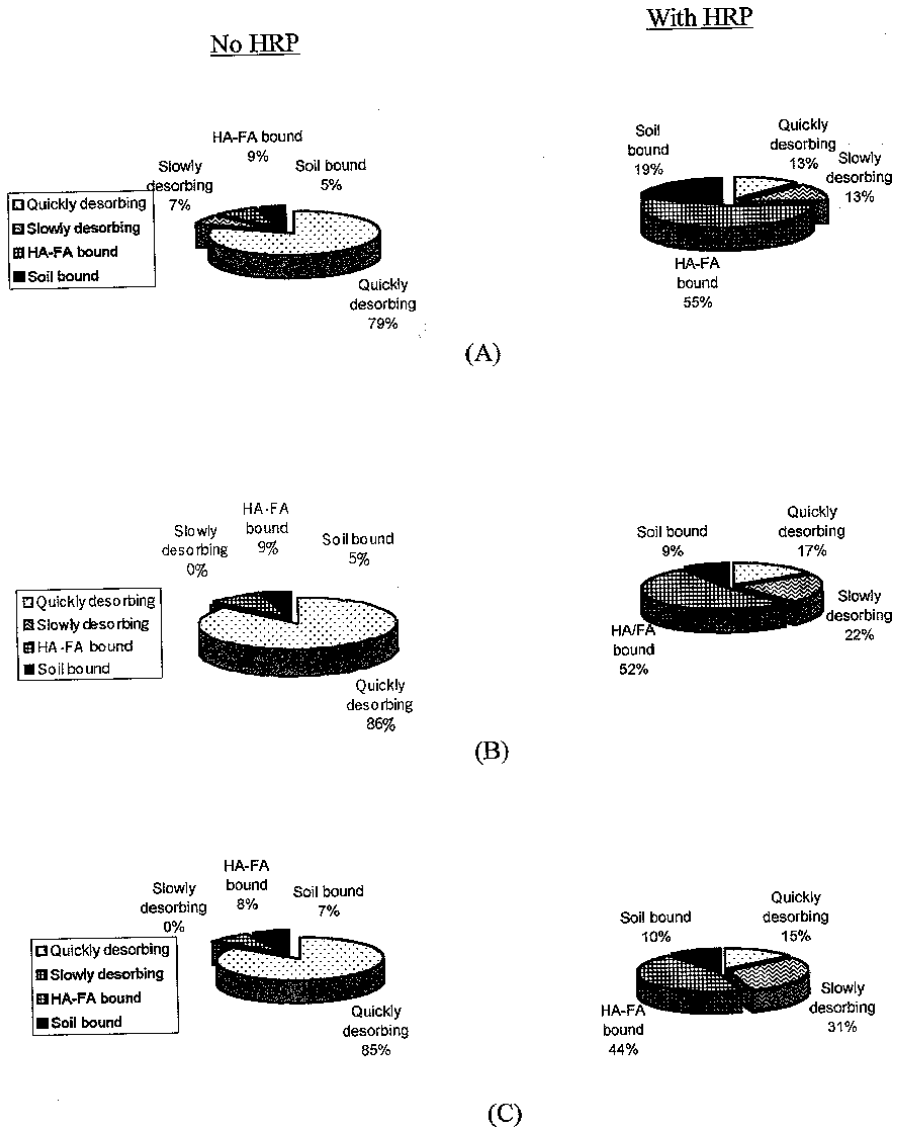

Figure 6. Distribution of phenol in field soil, in the abscence/presence of HRP, (a) present alone, (B) present with naphthol, (C) present with 2,4-DCP. The initial solute concentration is 50 μM.

Figure 6 represents data for the distribution of phenol in different soil fractions in the presence/absence of other phenolic chemicals. The initial solute concentration was 50 μM. In the absence of enzyme, more than 70% of phenol adsorbed was removed by water extraction, whether phenol was present alone or with co-contaminants. Compared with the distribution of DCP, it is obvious that DCP was harder to remove once it was adsorbed. Approximately 9% of adsorbed phenol was associated with humic/fulvic acids and 5~7% was bound to soil humin fraction in the absence/presence of co-contaminant. Because no enzyme was added in these cases and the binding occurred in a sterile system, it is believed that 1) the binding resulted from entrapment of chemicals within microporous regions of the SOM or mineral domains, or 2) other factors such as transition metal oxides may have

been responsible for the observed binding. In the case of DCP, almost no binding to humic/fulvic acids and soil humin fraction was observed without addition of HRP enzyme. Electron-withdrawing substituents (–Cl) in DCP are believed to have affected the binding of DCP to humic/fulvic acids and soil humin fraction because oxidative coupling reactions are electron-donating processes and more unlikely to occur with electron-withdrawing substituents. In the case of naphthol, even without addition of enzyme, most of adsorbed naphthol could not be removed by water or methanol extraction. A possible reason may be that methanol is an ineffective solvent for naphthol.

Upon the enzyme addition, the percentage distribution of phenol was altered, whether phenol was present alone or with other pehnolic chemicals. Less than 15% of adsorbed phenol was removed by water extraction and a higher percent of adsorbed phenol was removed by methanol extraction compared to when no HRP was added. More than 50% of adsorbed phenol was associated with humic/fulvic acids and soil humin fraction. In the case when phenol was present alone, 55.6% of adsorbed phenol was associated with humic/fulvic acids and 19% was bound to soil humin fraction. The presence of a co-contaminant decreased to a small extent the fraction of phenol associated with humic/fulvic acids and soil humin fraction. In the case of DCP present with phenol, however, there was 44% of adsorbed phenol associated with humic/fulvic acids and 10% percent bound to soil humin fraction. When naphthol was present with phenol, 53% of adsorbed phenol was associated with humic/fulvic acids and 9% was bound to soil humin fraction.

CONCLUSION

The investigation of horseradish peroxidase-mediated binding of phenol, DCP, and naphthol on field and forest soils indicates that the effect of addition of HRP on adsorption of phenolic chemicals depends on the hydrophobicity of the chemical. The more hydrophobic the chemicals are, the less effect the addition of enzyme has. The addition of enzyme altered the distribution of chemicals in soil fractions. Major portions of target chemicals adsorbed could not be removed by water or solvent extraction but remained bound to humic/fulvic acids and soil humin fraction.

The presence of co-contaminant did not influence the adsorption/desorption of phenol or DCP, whether enzyme was added or not. Enzyme addition increased the percentage of target chemicals bound to humic/fulvic acids and soil humin fraction, which was similar to the single chemical studies. The presence of co-contaminant, however, decreased the fraction of target chemicals associated with humic/fulvic acids and soil humin fraction.

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