Containment of Phenolic Contaminants in Soils by Peroxidase Addition

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Abstract: Adsorption-desorption and binding behaviors of several phenolic chemicals (phenol, *o*-cresol, 2,4-dichlorophenol and 1-naphthol) were studied on two sandy loam soils collected from field and forested sites. ^{14}C-labeled chemicals were used to track the distribution of the contaminant in the soil matrix. The effectiveness of horseradish peroxidase enzyme in enhancing adsorption and reducing desorption was evaluated for various soil-chemical combinations. Adsorption of single solutes and binary mixtures was studied. The degree of adsorption and binding was determined by quantifying the water extractable, solvent extractable, alkali extractable and soil-bound fractions. Addition of the enzyme resulted in dramatic increases in sorption and binding of phenol, cresol and dichlorophenol in single and binary solute systems. Desorption was little or negligible for these chemicals and hysteresis was significantly enhanced upon enzyme addition. A large amount of the radiolabel was associated with the humic/fulvic acid and soil/humin fractions. Containment of the target contaminant in soil was attributed to the production of strongly hydrophobic polymers in the aqueous phase and new "organic matter" on the soil.

Soils contaminated with phenols pose a high risk to ecosystem health because of the multiple toxic effects associated with these chemicals at very low concentrations. Anthropogenic phenols enter the soil environment as a result of accidental spills and uncontrolled discharges; they may also accumulate as intermediates during the incomplete biodegradation of aromatic compounds and pesticide mixtures. 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. Although sorption processes between organic chemicals and soil components have been well-studied chemical reactions between these pollutants and soil/sediment matrices have not been investigated in great detail. There is increasing evidence that chemical interactions between organic pollutants and soil components, specifically reactions catalyzed by extracellular soil enzymes, can significantly affect the fate of contaminants in soils and sediments, and potentially alter the associated health-risks from the chemicals. The work presented here represents results obtained from a comprehensive investigation of enzyme catalyzed binding of phenolic contaminants and their mixtures in two surface soils. Data obtained from these studies can be used to design engineered remediation systems that exploit the oxidative coupling properties of phenolic contaminants to contain them in soil and the subsurface.

Two surface soils were collected near the city of Manhattan in Riley County, KS. The soils were obtained from an agricultural field (SOM = 4.1%; pH = 7.3) and an adjacent forested site (SOM = 5.1%; pH = 7.1) and were classified as fine sandy loams. Soil was autoclaved at 115 ºC and 20 psi for 1 hour and transferred into an incubator set at 37 ºC for 2 days. The autoclaving and incubation procedure was repeated and followed by a final autoclaving to

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complete the sterilization procedure. When mixed with water, both soils were observed to release natural organic matter into solution. The soils were prewashed with a synthetic groundwater (GW) solution (pH 7 phosphate buffer + 500 mg/L sodium azide, 18 mM ionic strength) to remove all leachable SOM. The washed soils were dried at 70°C and homogenized with a mortar and pestle. The SOM content of the washed soils was 2.5% and 3.7% for the agricultural and forest soils.

The target chemicals used in this study included phenol, *o*-cresol, and 2,4-dichlorophenol. ^{14}C-radioisotopes were used to lower detection limits and track the distribution of the chemicals in the soil system. Nonlabeled target chemicals were added from concentrated stock solutions prepared in methanol. These solutions were then spiked with precise volumes of the corresponding radiotracer to obtain working solutions containing the desired amount of radioactivity. In studies involving mixtures, parallel experiments were conducted with one target chemical being radiolabeled while the other chemical was nonlabeled. Radioactivity in aqueous samples was enumerated as disintegrations per minute (dpm) using a Beckman 6500 liquid scintillation counter (LSC). Horseradish peroxidase (Type II, RZ: 2.2) and hydrogen peroxide (30% w/w) were obtained from Sigma Chemicals and used without further purification.

Adsorption studies were conducted in 16 mL-glass centrifuge tubes using 5 g soil in contact with approximately 14 mL of solution. The initial aqueous concentrations (C_0) of the target chemicals were 5, 50 and 500 μM. The treatments evaluated included: a) solution only (designated as *Control*), b) solution + soil (designated as *No Enzyme*), or c) solution + soil + peroxidase + H_2O_2 (designated as *Enzyme*). Triplicate sets of tubes were prepared for each concentration + treatment combination. Two AUs of peroxidase per mL of solution were added to tubes designated "Enzyme". Sufficient H_2O_2 was added to achieve a solution concentration equal to that of the target chemical. The tubes were allowed to equilibrate for 7 days at room temperature (22 $\pm$ 1ºC) in a tumbler. After 7 days, the contents of the tubes were centrifuged and the aqueous phase analyzed for the target chemical. Solid phase loadings were determined by mass balance.

After completion of the adsorption experiment, the supernatant from the centrifuge tubes was removed by pipeting and replaced by uncontaminated GW solution. All transfers were recorded in terms of volume and mass of fluid added or removed from each tube. The tubes were arranged in a rack and placed in a tumbler to mix for 24 h at 22 $\pm$ 1ºC. Tubes were removed from the tumbler on the following day, their contents were centrifuged and the supernatant was sampled for the radioactivity in solution resulting from desorption of the target chemical. The remaining supernatant was removed and the tubes refilled with uncontaminated GW. This "fill-and-draw" procedure was repeated until the radioactivity measured in the supernatant fell below the background radiation level of 50 dpm.

Extractions with GW were followed by sequential methanol extractions. The "fill-and-draw" solvent extraction procedure was repeated till the activity measured in the supernatant fell below 50 dpm. The GW and solvent extracted soils were subjected to base extraction with 0.1 N NaOH under a nitrogen. The "fill-and-draw" procedure was also used for base extractions. Sequential base extractions were continued until the supernatant appeared visibly clear indicating that all humic and fulvic acids had been extracted. The radioactivity remaining on soil after base

extraction was quantified by combusting the soil at 925 ºC in a Biological Oxidizer OX-500 (R.J Harvey Instrument Co., New Jersey). Combustion resulted in the conversion of all ^{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., New Jersey) and quantified on the LSC.

Results from this study demonstrate the effectiveness of peroxidase in reducing the mobility of phenolic contaminants in surface soils. This reduction in mobility is brought about by the polymerization of phenols and subsequent adsorption of the polymers on soil particles. The large size and low aqueous solubilities of the polymers prevent these macromolecules to desorb readily. It is also likely that enzyme mediated oxidative polymerization may have resulted in the direct cross-linking of the target compounds to SOM. Such reactions can attenuate the migration of phenolic pollutants in soils by reducing contaminant transport with surface runoff, or leaching into the subsurface. The more soluble and, therefore, more mobile phenols are affected to a greater extent by the peroxidase mediated immobilization technique. Figure 1 is a conceptual diagram illustrating the application of enzyme-mediated polymerization processes to immobilize phenolic chemicals and thereby contain contaminant plumes in the subsurface. Addition of enzyme, may be accomplished through an injection well or by constructing a permeable reactive barrier with immobilized enzymes. Enzymes immobilized on clays and soils can retain their ability of mediating oxidative coupling processes over long periods. Addition of enzyme to the contaminated water would result in polymerization of phenols and their subsequent adsorption or binding to soil or aquifer solids. Polymerization and binding of the phenols can produce a significant reduction in contaminant bioavailability. Bioavailability reductions appear to arise from the low aqueous solubility of the polymers produced. Chemical alterations such as the fortuitous dechlorination of chlorophenols during polymerization may also lead to lowered toxicity.

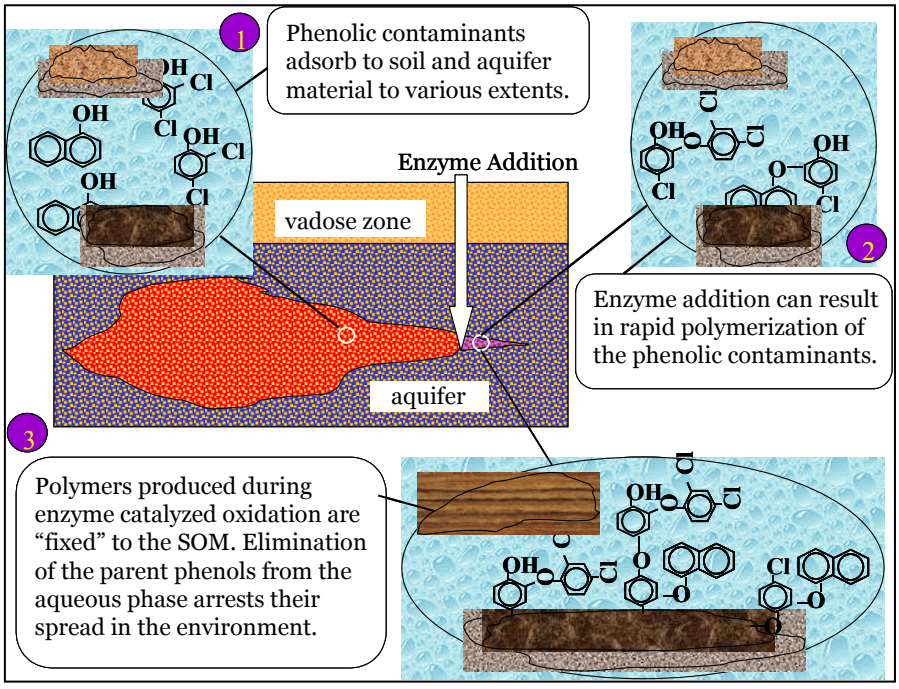

Figure 1. Conceptual diagram illustrating application of enzyme to immobilize phenols.