INVESTIGATION OF THE INTERACTIONS AMONG
GRASS, CHLOROPHENOLS AND MICROBES

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ABSTRACT

Studies were conducted to explore the interactions among rye grass, chlorophenols and microorganisms. The objectives were to examine some of the processes by which plants affect the fate of subsurface organic contaminants. The research was divided into three studies: interactions between live grasses and 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP), and pentachlorophenol (PCP); physico-chemical interactions between the three chlorophenols and root tissue; and effect of root exudates on biodegradation of TCP.

To study the interactions between plants and organic contaminants, rye grass plants were grown in solutions containing DCP, TCP or PCP for one to three weeks. The grass removed substantial amounts of the chlorophenols throughout the incubation time. The majority of each chlorophenol removed from solution could not be recovered by non-destructive solvent extraction. The removal of the chlorophenols from solution and the unrecoverability of the removed compound followed different kinetics, indicating that the two are different processes. Both contaminant removal and unrecoverability were closely related to root surface area but not to transpiration. A qualitative model was developed to describe the uptake of organic contaminants by plants. The data demonstrate the importance of physico-chemical interactions between contaminants and roots and suggest that maximization of root surface area should be one consideration when selecting a plant species for phytoremediation.

To study the physico-chemical interactions between plant roots and organic contaminants, the distribution of DCP, TCP and PCP within a three phase
system was examined. The three phases were severed grass roots, water and an organic solvent, either hexane or ethyl acetate. The chlorophenol mass that partitioned into the solvent phase was inversely correlated with root mass and root surface area index. Partition coefficients calculated with respect to the organic liquid phase were inversely correlated with root mass and root surface area index. A similar partitioning experiment was conducted using PCP placed in a solution containing only the dissolved organic material released by roots. These resulting partition coefficients decreased with increasing organic carbon concentration. It appeared that the organic compounds released into solution by the roots affected the movement of the chlorophenol into the organic liquid phase. It is proposed that the presence of roots simultaneously promoted retention of the chlorophenols in the aqueous phase and provided a sorption site.

The effect of grass root exudates and glucose on the lag time associated with 2,4,6-trichlorophenol (TCP) degradation by an unacclimated microbial inoculant and an acclimated microbial inoculant was investigated. The presence of an alternate organic carbon source reduced lag time for both the acclimated microbial inoculant and the inoculant that had not been previously exposed to chlorinated phenols. The lag time for acclimation of microbes to TCP mineralization was affected by the ratio of the alternate organic carbon source concentration to the biomass concentration. It is proposed that the presence of a readily available, alternate organic carbon source affected lag time through promotion of microbial population growth and provision of a preferred source of carbon and energy.

The results indicate that rye grass may directly, through partitioning and uptake, and indirectly, through soil microbes, affect the fate of chlorophenols in the subsurface environment.
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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Contamination of soil with organic compounds is a widespread problem. Due to its less invasive nature, in situ remediation of a contaminated site offers certain advantages over excavation, treatment and disposal of the soil. One in situ approach that has gained attention is the reliance on naturally occurring soil microorganisms to convert organic contaminants to cell biomass, carbon dioxide, water and elemental constituents through enzymatic reactions, a process also known as bioremediation. This process may be inhibited by several factors, such as the nutrient and redox conditions of the soil and the association of the contaminants with soil organic matter. In practice, in situ bioremediation may not rapidly and/or completely reduce pollution at a given site to acceptable levels. Therefore, it is important for the scientific community to develop methods by which bioremediation can be rendered more effective.

One method that has gained the attention of the research community is the addition of plants to a contaminated site. In the past decade, much research has focused on the ability of plants to accumulate metals in their tissues. Recently, some studies have considered the effects of plants on organic contaminants. The agricultural literature is replete with studies that assess crop plants' and weeds' abilities to internalize and metabolize organic pesticides. From this point, it is logical to hypothesize that plants are also capable of taking up and metabolizing non-pesticide xenobiotics. At the least, the water flux generated by the transpiration process should affect the mobility of the contaminants within the soil.

In addition, plant roots create a zone of enhanced microbial activity called the rhizosphere. Bioremediation relies on the ability of microbial enzymes to use the xenobiotics as a reaction substrate. In a soil system, plants comprise the majority of the organic material upon which the microbes would
normally feed. Plant tissues contain a wide variety of complex organic chemicals which promote the synthesis of microbial enzymes capable of using these chemicals. Since plants stimulate microbial activity, and since plants also contain organic compounds that are similar in structure to many xenobiotics, it is logical to hypothesize that the presence of plants on a contaminated site will affect the microbial activity and hence the microbial metabolism of the contaminants.

It is the intent of this research to identify mechanisms by which plants affect, directly and indirectly, the fate of organic contaminants in the environment.

THE RHIZOSPHERE

The rhizosphere is the area of soil that immediately surrounds the roots and is influenced by the roots (Tate III, et al., 1991). This region is characterized by greater numbers of microbes, greater species diversity, and greater microbial activity (in terms of organic matter consumption) than the soil beyond the rhizosphere (Tate III, et al., 1991). This effect results from two aspects of plant roots. First, they provide a surface to which microorganisms can adhere. Second, the roots of a living plant do not simply allow the influx of soil water and its dissolved constituents, they actively release specific chemicals and passively "leak" other organic compounds into the surrounding soil. Sloughing of tissue as the roots penetrate the soil also contribute to the plant-derived pool of organic compounds. The organic molecules released to the soil range from simple sugars and amino acids to complex polymers. The specific chemicals and their quantities released by the plant depend on the species, developmental stage and environmental stress (Tate III, et al, 1991). This material provides carbon and energy for the microorganisms attached to and proximate to the roots, thereby stimulating microbial activity.

The ability of roots and root-derived material to stimulate microbial degradation has been demonstrated in several experiments. Hsu and Bartha
(1979) observed that mineralization of parathion and diazinon was greater in the presence of roots than in an unplanted soil. In addition, mineralization of parathion in an unplanted soil was stimulated when the soil was watered with exudates from a bush bean plant. In a study by Federle and Schwab (1989), two surfactants, linear alkylbenzene sulfonate and linear alcohol ethoxylate, were mineralized more quickly and to a greater extent in the presence of living cattail roots and absence of sediment than in the presence of sediment and absence of roots. Boyle and Shann (1995) found that 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid were mineralized at higher rates in rhizosphere soils as compared to similar non-rhizosphere soils. Walton and Anderson (1990) observed similar results for the biodegradation of trichloroethylene in rhizosphere and non-rhizosphere soils.

It has been hypothesized that the root-derived organic matter stimulates the degradation of xenobiotics by two mechanisms: provision of growth substrates for microbes that increases the number of microorganisms in that area; and release of compounds structurally similar to the xenobiotics, causing the microbial community to shift in composition towards microbes that are capable of degrading these chemical structures (Boyle and Shann, 1995; Ferro, et al., 1994). Research indicates that the presence of plant roots supports a microbial community capable of metabolizing more structurally complex natural organic matter than a non-rhizosphere community (Helal and Sauerbeck, 1986). However, it is likely that the specific effect roots may exert on contaminant mineralization depends on the chemical of concern.

In addition to the effects on the microbial community, plant roots influence the physical characteristics of the surrounding soil. Except for some hydrophytic species, such as cattails, plants consume oxygen present in the soil water, competing with aerobic soil microorganisms for a finite amount of oxygen. During the day, transpiration generates negative water potentials in the rhizosphere. At night, while the stomata are closed, the soil water potential increases. Thus, rhizosphere microbes must be able to live under conditions of
rapidly changing water potentials. The physical effects of roots on microbes have not yet been elucidated.

**PLANT UPTAKE AND METABOLISM**

The plant roots may also directly affect the fate of organic contaminants. Once in the soil, a portion of an organic contaminant remains in the aqueous solution while the rest associates with the soil organic matter. The exact nature of this association with the organic matter depends on the specific chemical and results from four mechanisms: liquid-liquid partitioning; sorption; hydrophobic interactions; and oxidative coupling (Crane, 1992; Berry, 1984). The organic carbon partition coefficient is used to describe the division of the total quantity of contaminant between the organic material and the aqueous phase. The fraction of the organic contaminant dissolved in the soil water is available to the plant for transpirational uptake.

Sorption, hydrophobic interactions and oxidative coupling may also occur between the roots and organic contaminants. The root surface is comprised of a multitude of different organic chemicals, including enzymes. Perhaps some plant species contain peroxidase on the root exterior, allowing the oxidative coupling of phenols and anilines to the root surface. Xenobiotics may be able to adsorb to specific sites on the root epidermis. In addition, the root provides an organic surface along which hydrophobic contaminants can aggregate out of the bulk aqueous solution.

Pesticide research has generated a large body of data concerning the uptake of externally applied organic compounds by plants. Pesticides encompass a wide variety of chemicals, many of which are similar in nature and structure to the xenobiotics that are commonly treated through bioremediation techniques. According to Hance (1988), most herbicides in the soil enter the plants as solutes in the soil water that moves into the root apoplast. Once within the root epidermis, the compound may associate with the plant tissue via
partitioning or adsorption, may diffuse throughout the tissue water, or may cross cell membranes and enter the symplast. Devine and Born (1988) state that, while some herbicides may be transported into the plant entirely in the apoplast, through breaks in the casparian strip, most herbicides move beyond the endodermis via the symplast. Since it is unlikely that plant cells have evolved active transport enzymes for organic chemicals, symplast entry requires these chemicals to be able to diffuse across cell membranes. Hence, the polarity of a contaminant controls its uptake by the root. It has been found that dissociable chemicals exhibit greater absorption when the pH favors the undissociated form (Devine and Born, 1988).

Briggs, et al (1982) observed that the uptake of nonionizable organics by barley decreased with decreasing lipophilicity to a minimum root concentration factor that remained constant regardless of the degree to which contaminant lipophilicity further decreased. Their data suggested that two mechanisms governed root uptake: one mechanism that was a function of contaminant hydrophobicity; and one mechanism that was not a function of chemical structure. The authors attributed this latter mechanism to equilibration of the external solution with the water contained in the free space and cells via diffusion. The authors concluded that the former process was partitioning to the root solids.

Once inside the root, translocation to the other parts of the plant is a function of polarity: "there is an optimum lipophilicity for maximum translocation to shoots, centered at log $K_{ow} = 1.8$" (Briggs, et al, 1982, page 501). Very polar compounds cannot permeate membranes, and hence cannot move through the casparian strip and into the xylem. Very nonpolar compounds readily permeate the cell membranes, but are unable to redissolve into either the apoplastic or symplastic water, causing the compounds to remain associated with the cell membranes and cell walls.
Within the plant tissues, the majority of herbicides undergo some type of enzymatically mediated reaction (Hatzios, 1988). The metabolism of organic contaminants by plants is generally divided into three phases:

"The primary or phase I reactions include mainly nonsynthetic processes such as oxidations, reductions, and hydrolyses, while secondary or phase II reactions, often referred to as conjugations, are synthetic reactions. Phase I metabolism often results in the formation of metabolites with reduced or modified phytotoxicity, increased polarity, or predisposes the parent molecules for further metabolism in the secondary phase. Conjugations of xenobiotics in phase II metabolism result in the formation of metabolites with greatly reduced or no phytotoxicity, higher water solubility, and limited mobility . . . In phase III, herbicide conjugates formed in phase II are converted to secondary conjugates or insoluble bound residues such as lignin biopolymers." (Hatzios, 1988, Page 144)

The following enzymatic reactions have been documented to occur with pesticides in plant tissue: N-dealkylation; O-dealkylation; N-deamination; aromatic hydroxylation; thioether oxidation; nitrogen oxidation; \( \beta \)-oxidation of phenoxybutyric acids; species specific instances of reduction; hydrolysis of esters, amides and nitriles; glycosidic conjugation; glutathione conjugations; and amino acid conjugation (Hatzios, 1988). Casterline, et al (1985), observed the uptake and subsequent transformation by soybean and spinach of PCP into multiple metabolites, including tetrachlorophenol, trichloroanisole and O-glucosides. Schmitt, et al (1985) documented the formation of \( \beta \)-D-glucosyl and O-malonyl-\( \beta \)-D-glucosyl conjugates from the uptake of PCP into soybean cell cultures. Edwards (1986) monitored the uptake of radioactive anthracene by bush bean plants from a nutrient solution. After 30 days of contact with the plants, less than one fifth of the radioactivity remained in solution and approximately the same amount of activity could not be accounted for. Of the activity taken up by the plant, the majority remained in the roots and only a small fraction was translocated to the shoots. Approximately three quarters of
the activity within the plant tissue represented some type of anthracene metabolite, grouped into nonpolar, polar and nonextractable compounds.

The above studies demonstrate that, if an organic contaminant is present in the soil solution, plants have the ability to bring the chemicals into their tissues and to transform the xenobiotics. The degree to which this process occurs in the environment depends on several factors, including: contaminant hydrophobicity, the amount of soil organic matter available for adsorption, the rate of transpiration, the plant species, and the activity of microbes capable of degrading the compound before it reaches the plant roots. This research will consider the role of plant uptake in phytoremediation.

OTHER POTENTIAL MECHANISMS

The association of xenobiotics with soil organic matter renders the contaminants inaccessible to microbial degradation (Robinson, 1990). To ensure rapid removal of the subsurface contaminant, it is necessary to promote desorption. Often, desorption is slow and in some cases the association with humic material is an irreversible, or very slowly reversible, reaction (Crane, 1992). Through transpiration, a group of plants on a contaminated site can generate a flux of cleaner water from the periphery into the contaminated zone, enhancing desorption through flushing. Plants should also minimize leaching of xenobiotics into less or un-contaminated areas.

In some situations, the ability of the soil microbes to degrade the contaminants is limited by the nutrient status of the soil. Nitrogen and phosphorus are macronutrients that could limit microbial growth. Due to its highly insoluble nature in the soil matrix, phosphorus may not be in a form that microorganisms can use. Iron is another element essential for microbial growth that may be present in limited quantities.

In some soils, lack of nitrogen, phosphate and iron can limit plant growth. A number of plant species have adapted themselves to living under
these nutrient stress conditions by using their roots to solubilize the often immobile nutrients phosphorus and iron. For example, white lupin is able to solubilize phosphate and iron in excess of the plant's own requirements through the release of citric acid (Marschner, et al., 1987). Bar-Yosef (1991) outlined several mechanisms by which root exudates affect the solubility of phosphate: pH alterations; surface charge alterations; formation of stable complexes; and layer silicate platelet collapse. Root exudates contain organic acids, protons and hydroxyl anions. The effects on the pH of the soil surrounding the roots can be dramatic. "Rhizosphere pH may differ from the bulk soil pH by more than 2 units, depending particularly on the nitrogen source and plant species and the bulk soil pH" (Marschner, et al., 1987). The solubilities of the phosphate minerals are highly pH dependent.

Specific adsorption is the process by which phosphate strongly associates with the edges of layer silicates and aluminum/iron oxides. Other anions have the ability to compete for these sorption sites. Nagarajah, et al (1968) observed reduced sorption of phosphate onto kaolinite in the presence of citrate, a common root exudate, and bicarbonate (roots release CO$_2$). According to the authors, "the reduction in phosphate adsorption must result not only from some competition but also from a concomitant change in charge due to citrate or bicarbonate adsorption." The data from Nagarajah, et al (1968) exhibited a marked desorption of phosphate after the introduction of citrate to the clay solution. Many other researchers have noticed increased solubilization of phosphate in the presence of organic anions (Earl, et al, 1979).

The ability of root exudates to complex calcium, iron and aluminum could influence phosphate solubility (Kepert, et al, 1979). By reducing the concentration of free metal (such as iron) in solution, the stable complexes formed by the organic anions would promote dissolution of phosphate minerals. Gardner, et al (1983) hypothesize that the formation of a citrate/Fe/OH/H$_2$PO$_4^-$ polymer enhances the movement of both phosphorus and iron to the roots. The authors propose these polymers to be very stable in the soil and to be degraded
by the reducing activity of the roots. The paper implies that, once the polymer has been broken down, the plant roots would take up the released phosphate. However, since the reaction appears to occur on the exterior of the root, it is possible that microbes of the rhizoplane could compete with the plant for the phosphate.

Plants have two primary strategies to increase the availability of iron: release of protons and reductants in the root exudates with a simultaneous increase in reductase activity, used by dicots and non-graminaceous plants; and release of phytosiderophores by graminaceous species (Awad, et al, 1994). It is not clear if the iron chelated by phytosiderophores is available to microbes that degrade organic contaminants. Gardner, et al (1987) linked citrate with the formation of Fe/OH/H₂PO₄⁻ polymers that facilitated the transport of iron to the roots. At the root surface, plant reductants release the iron from the polymer and transport it into the root. Whether or not rhizosphere microbes can prevent the solubilized iron from reaching the plant roots has not been determined.

To overcome nitrogen deficiencies, certain plants and microorganisms have developed symbiotic relationships in which the plants provide the organism with readily oxidizable carbon while the organisms fix dinitrogen gas into a plant available form. By not using the nitrogen already present in the soil, this plant-microorganism symbiosis increases the soil nitrogen content, although the nitrogen may not be available to other organisms until the plant dies or loses some of its biomass through leaf drop. Nitrogen fixation contributes 8.5% of the global total plant uptake per year (Paul and Clark, 1989). Certain plants, such as the water fern Azolla and clover, have been used extensively in agriculture for hundreds of years due to their ability to maintain soil fertility. The Cyanobacterium associated with Azolla can add between 30 and 70 kg.ha of nitrogen per year (Paul and Clark, 1989). The potential benefits of nitrogen fixation on nonsymbiotic-microbial growth, and thus microbial degradation of xenobiotics, would result only after the bound nitrogen has been released into
the soil nutrient pool. Use of nitrogen fixing plants to enhance bioremediation would require a multi-crop or multiple growing season approach.

SUMMARY

A review of the literature from a variety of fields suggested several different mechanisms by which plants could affect the fate of organic contaminants. The mechanisms can be divided into two categories: direct effects and indirect effects. The plant might directly affect organic contaminants through root uptake and metabolism, enhanced desorption from the soil organic matter by the flushing action of transpiration, and minimization of off-site transport also by the hydraulic effects of transpiration. Indirectly, the plant may stimulate microbial degradation of xenobiotics through the release of organic material from the roots and the improvement of a soil’s nutrient status. Plant roots affect the physical soil structure, the redox conditions, and the water potential of the surrounding soil. These factors also influence microbial activity.

It is not possible to examine all of the identified mechanisms in a single dissertation. Therefore, this research will focus on three areas: the effects of soluble root products on microbial degradation of xenobiotics; the association of organic contaminants with plant roots; and the direct uptake of xenobiotics by plants.

REFERENCES


CHAPTER 2: ROLE OF PHYSICO-CHEMICAL PROCESSES IN CHLOROPHENOL UPTAKE BY RYE GRASS

By Cynthia E. Crane and John T. Novak

ABSTRACT: To study the interactions between plants and organic contaminants, rye grass plants were grown in solutions containing either 2,4-dichlorophenol, 2,4,6-trichlorophenol, or pentachlorophenol. Within one week, the grass had removed substantial amounts of the chlorophenol from solution. Contaminant removal continued for the duration of the study. After sacrifical sampling, the roots were extracted with ethanol. The majority of each chlorophenol removed from solution could not be recovered by non-destructive solvent extraction. The mass that could be recovered from the roots decreased with increasing duration of root exposure to the chlorophenol. The removal of the chlorophenols from solution and the unrecoverability of the removed compound followed different kinetics, indicating that the two are different processes. Both contaminant removal and unrecoverability were closely related to root surface area but not to transpiration. Removal of each chlorophenol from bulk solution was well described by the Langmuir isotherm. Finally, transpiration was not required for either contaminant removal or unrecoverability. Based on these results, a qualitative model was developed to describe the uptake of organic contaminants by plants. The data demonstrate the importance of physico-chemical interactions between contaminants and roots. The results suggest that maximization of root surface area should be one consideration when selecting a plant species for phytoremediation.

Key Words: chlorophenol, phytoremediation, uptake, rye grass, remediation, roots
INTRODUCTION

The use of plants to treat contaminated soil is a promising technology. Research has shown that plants have the ability to take up organic pesticides from the soil, translocate the compounds from the roots to the shoots, and metabolize the compounds (Briggs, et al., 1982; Devine and Born, 1988; Hance, 1988; Hatzios, 1988). However, several issues require further exploration in order to effectively design plant systems for in situ remediation (Cunningham, et al., 1996). For example, the extent of the role that plant uptake may play relative to other influences that plants exert on in situ remediation, such as enhancement of contaminant biodegradation, is unknown.

Much of the recent research has focused on the fate of a compound once inside a plant (Burken and Schnoor, 1997; Schnabel, et al., 1997). Before a plant can translocate or metabolize a contaminant, the compound must be brought inside the root tissue. This research examined the manner in which plants remove a contaminant from the surrounding solution. The purpose was to assess the effectiveness of plants in removing an organic contaminant from a system and to determine mechanisms by which this removal occurs.

METHODS AND MATERIALS

Plant uptake studies were conducted using 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP) individually in solution. The three chlorophenols were selected in order to provide a range of hydrophobicities (log octanol-water partition coefficient ($K_{ow}$) of 2.75, 3.38 and 5.01 for DCP, TCP and PCP, respectively, Westall, et al., 1985) using compounds of similar molecular structure. The similarity in molecular structure minimized variability in potential enzymatic reactions of the compounds within the plant tissue. TCP was studied using an initial concentration of 0.049 mM. Due to toxicity concerns, the initial concentrations for PCP and DCP were halved to 0.024 mM. Rye grass was selected as the experimental plant for its ease of growth within the laboratory and its potential for use in the field. In order to
isolate the interactions between the plants and the test compounds, all experiments were conducted in a hydroponic system.

**Experimental Procedure**

Rye grass seeds from a commercial mix were germinated in 70 mL, straight-sided, glass jars filled with semisolid bactoagar (6 grams bactoagar/liter dilute nutrient solution). After germination, the jars were placed under cool, white lights and the seedlings were allowed to grow for three weeks prior to experimental use. The bactoagar was sufficiently dilute to allow the plants to obtain water and nutrients directly from the agar without the addition of nutrient solution. The photoperiod was 14 hours.

Fifty mL of chlorophenol/nutrient solution were pipetted into 70 mL, straight-sided glass jars. The jar walls were covered with aluminum foil. A small hole was cut into a square of parafilm, and the parafilm was placed across the top of the jar and secured with tape. Next, grass seedlings were removed from the agar and rinsed with autoclaved water, then cotton was wrapped around the shoots slightly above the roots. The roots were inserted through the hole in the parafilm and the cotton was taped onto the parafilm to provide a means of anchoring the plants in place. Each jar was checked to ensure that the roots were immersed in the solution. The number of plants in each jar were varied in order to determine which variables, such as root mass and transpired volume, were most closely related to compound removal. Each sampling episode for each compound consisted of jars containing from ten to forty seedlings and three jars containing no plants. These plantless jars were set up in the exact same manner as the plant-containing jars except that cotton was taped directly over the hole in the parafilm. The plantless samples served as controls to monitor system loss.

Replicates were sacrificed weekly up to three weeks for TCP and PCP, and up to two weeks for DCP (due to volatility of DCP). At sampling, the shoots were clipped from the roots with scissors and weighed. Next, the roots were weighed, placed in screwtop vials and extracted with ethanol for 24 hours. After extraction, the root surface area index was determined using the procedure.
described below. The chlorophenol content of the ethanol extract was analyzed by gas chromatography. To demonstrate that the plants, and not suspended microbes, were responsible for the chlorophenol loss, after the plants were removed each flask was spiked with the appropriate chlorophenol, sampled immediately and resampled 24 hours later. Except for one TCP flask from week 2, for which the data were not used, none of the spiked samples yielded chlorophenol loss during the 24 hour incubation, indicating that microbial metabolism was not likely to have influenced the results.

The aqueous samples were extracted with hexane. Five mL of each aqueous sample was placed into an 11 mL screwtop vial and acidified to below pH 2 with 4 drops of 1 N sulfuric acid. For TCP, 3 mL of hexane were pipetted onto the aqueous phase, and the two phases were mixed by wrist action. The phases were allowed to separate and the hexane was removed from the aqueous sample with a Pasteur pipette and placed into another screwtop vial. This process was repeated twice. Finally, one mL of the hexane extract was placed into an autosampler vial and the remaining hexane volume was measured. For DCP and PCP, 3 mL of hexane was pipetted onto the aqueous phase. The two phases were allowed to contact for 24 hours, after which the hexane was removed by Pasteur pipette. Next, one mL of the hexane extract was placed in an autosampler vial and the remaining hexane volume was measured.

All of the extracts were analyzed on a Hewlett Packard 5890A Gas Chromatograph fitted with an electron capture detector, an autosampler and a Supelco DB5 column. The aqueous extraction standard deviations were ±12.1% for DCP (n = 30), ±9.09% for TCP (n = 55), and ±6.89% for PCP (n = 25).

**Extraction of TCP from Roots**

In a side study examining the sorption of TCP to grass roots, the extractability of the radiolabeled TCP that had sorbed to severed roots was measured. Roots were placed in 20 mL of a dilute nutrient solution containing 5.04 mg/L TCP. There were 12 root-containing samples and 4 rootless controls to monitor system loss. The solutions were sampled after 3, 7 and 11 days. By day
3, the removal of TCP from solution was 98.5% complete (standard deviation of 0.819%), indicating that sorption was essentially complete within the first 3 days. On day 11, the roots from 6 samples were transferred into 10 mL of ethanol (the remaining 6 were extracted with water; data not considered here). After 24 hours, the roots were transferred to another 10 mL of ethanol and the ethanol extract was analyzed for radioactivity. This process was repeated 3 times. After the last 24 hour extraction, the roots, in addition to the ethanol extract, were analyzed for radioactivity. Radioactivity was measured by placing the sample into 14.75 mL of Scintiverse BD and analyzing the sample on a Beckman LS-3150T (analytical error of 2.67%).

After the first 24 hour extraction period, an average of 43.3% of the sorbed TCP was recovered by the ethanol (standard deviation of 4.25 percentage points, or 9.81%, n = 6). The vast majority, 93.7%, of the TCP ultimately extracted by the above approach was recovered within the first 24 hour extraction period.

**Root Surface Area Index**

Measurement of the adsorption of rhodamine red by roots was used to determine relative surface areas of the samples (Sorenson and Wakeman, 1996). The roots were placed into 125 mL Erlenmeyer flasks containing 30 mL of a rhodamine red solution with a concentration of 2 µM and at a pH of 4.9. The flasks were agitated for 18 hours on a shaker table to achieve equilibrium prior to centrifugation at 10,000 rpm for 10 minutes. The absorbance of the centrate was measured on a spectrophotometer at a wavelength of 550 nm. The adsorbed mass, expressed as umoles, is referred to throughout the paper as the root surface area index and reflects the relative adsorptive capacity of the different samples.

**RESULTS AND DISCUSSION**

In the presence of live rye grass, the concentration of each chlorophenol in solution was substantially reduced within the first week, during which time minimal transpiration occurred (Figure 2-1). At the highest root content, more
than 50% of each chlorophenol was removed from solution. The loss of chlorophenols from solution appeared to be directly related to the amount of root surface area, suggesting that adsorption was responsible for the initial uptake by the plants.

![Graph](image)

Figure 2-1. Decrease in aqueous chlorophenol concentration with increasing root surface area, samples from one week incubation time.

The aqueous concentration continued to decrease with increasing incubation time. Data for TCP are presented in Figure 2-2. By the final sampling time, depending on the number of plants in the flask, up to 90% of the PCP mass, 95% of the DCP mass and greater than 99% of the TCP mass had been removed from solution.

For each test compound, most of the total mass removed from solution could be not extracted from the roots by ethanol. This is shown in Figure 2-3 where the extractable fraction for the three chlorophenols after one week of incubation are compared. The extractable fraction was calculated as a percentage of the total mass removed from solution. The extractable fraction of DCP was less than 10% for all samples and decreased only slightly from week one to week two (Table 2-1). About 11% of the TCP and 37% of the PCP was extractable after week one. As noted in the methods section, the average 24-hour ethanol
extraction efficiency for TCP sorbed to the roots of non-growing plants was 43.3%. Thus, while some of the TCP becomes non-extractable due to sorption to the roots, additional activity by the growing plants is responsible for the increased resistance to extraction. From week 1 to week 2, the extractable fractions for both TCP and PCP decreased substantially (Table 2-1) while from week 2 to week 3, little additional change occurred.

![Figure 2-2](image1.png)

**Figure 2-2.** Decrease in TCP aqueous concentration, shown as a percentage of initial concentration, for one week and three weeks incubation time.

![Figure 2-3](image2.png)

**Figure 2-3.** Extractable fraction of each chlorophenol after one week of incubation.
Table 2-1. Range of extractable fractions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCP</td>
<td>2.9% - 8.8%</td>
<td>2.4% - 9.2%</td>
<td></td>
</tr>
<tr>
<td>TCP</td>
<td>9.0% - 54%</td>
<td>1.1% - 3.4%</td>
<td>1.1% - 3.6%</td>
</tr>
<tr>
<td>PCP</td>
<td>20% - 55%</td>
<td>4.9% - 13%</td>
<td>6.9% - 12%</td>
</tr>
</tbody>
</table>

As was documented by Briggs et al. (1982), the movement of organic chemicals to the shoots, as indicated by the transpiration stream concentration factor (TSCF) was influenced by the $K_{ow}$ of the chemicals, with the maximum movement occurring at a $K_{ow}$ of 2 and decreasing as the $K_{ow}$ increased. Based on the results of Briggs, et al., DCP would be expected to move readily into the plants and be the least extractable while PCP would be expected to remain longer on the root surface. It is clear that the extractability, and hence recoverability, is affected by hydrophobicity. PCP also produced the greatest scatter.

Different plant mechanisms, such as metabolic transformations, translocation, and irreversible association with the root surface (such as through oxidative coupling) could account for the inability of ethanol to recover the majority of the contaminant removed from solution (Briggs, et al., 1982; Hatzios, 1988; Burken and Schnoor, 1997). In this study, the amount of each compound that could not be recovered, also known as the unrecoverable mass, was calculated by subtracting the sum of the chlorophenol mass extracted from the roots and the mass of chlorophenol remaining in solution from the mass present in the controls. The unrecoverable fraction was computed with respect to the mass in the controls at the time of sampling. After one week, the three chlorophenols produced similar unrecoverable fractions for similar root surface area indices (Figure 2-4). While DCP and TCP increased in an approximately linear manner with respect to root surface area index, PCP exhibited scatter (Figure 2-4). As the test period lengthened, the unrecoverable fraction increased for all three compounds.
The rate of removal from solution and the rate of unrecoverability did not appear to be the same. For example, at one week, the unrecoverable PCP mass ranged from 44.6% to 80.2% of the mass of PCP removed from solution, with an average of 62.9 ± 11.1%. It should be noted that these samples encompassed a range of root surface area indices, from 0.0139 to 0.0241 μmoles rhodamine red adsorbed. Over a similar range of root surface areas, at two weeks the unrecoverable mass had increased to 87.4% to 95.1% of the mass removed, with an average of 92.4 ± 2.61%. Normalizing the unrecoverable mass to the root surface area index clearly demonstrates the increase in the unrecoverable mass with time (Figure 2-5). At week 1, the unrecoverable PCP mass per unit root surface area ranged from 0.0102 to 0.0249 mmoles PCP/umole rhodamine red. At week 2, these values increased to between 0.0214 and 0.0442 mmoles PCP/umole rhodamine red. Meanwhile, the mass of PCP removed from solution normalized to the root surface area index was similar for weeks one and two (Figure 2-6). The increase in the unrecoverable fraction between week 1 and week 2 combined with the similarities in the amount removed at the same sample times indicates that unrecoverability and removal followed different kinetics. Similar but less pronounced trends were observed for TCP and DCP (data not
shown), suggesting that the processes rendered chlorophenols unrecoverable proceeded more rapidly for TCP and DCP than for PCP.

Figure 2-5. Unrecoverable PCP mass normalized to the root surface area index, incubation times of one, two and three weeks.

Figure 2-6. Mass of PCP removed from solution normalized to the root surface area index. Incubation times of one, two and three weeks.

The results for PCP are not inconsistent with those obtained by Burken and Schnoor (1998) using poplar cuttings. These authors limited their experiment
to approximately one week, and observed that, after initial sorption, additional 
PCP was not removed from solution. The rye grass results suggest that, during 
the first week, further removal of PCP beyond sorption may be limited by the rate 
of movement into the plant root system. Extending the study time beyond one 
week allows more PCP removal to occur as the sorbed mass becomes unrecoverable.

To determine which plant variables were most strongly related to 
chlorophenol distribution, correlation coefficients were calculated (Table 2-2 and 
Table 2-3). The root surface area index tended to yield the strongest correlations 
with the mass of chlorophenol in solution and the unrecoverable mass. Shoot 
mass, root mass and root surface area index were strongly correlated with each 
other. The strong correlation of shoot mass with root surface area and root mass 
explains the strong correlation of shoot mass with the distribution of the 
chlorinated phenols. Since the surface area reflects the adsorption area and since 
the root mass reflects the volume into which the compounds diffuse, removal 
should be related to both variables. The weaker correlations with root mass 
indicate that diffusion plays a relatively minor role as compared to adsorption. 
The most hydrophobic compound was characterized by the weakest correlation 
coefficients.

Table 2-2. Correlation coefficients for mass remaining in solution.

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Root Surface Area Index</th>
<th>Root Mass</th>
<th>Shoot Mass</th>
<th>Transpired Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCP, 1 week</td>
<td>-0.949</td>
<td>-0.796</td>
<td>-0.850</td>
<td>-0.739</td>
</tr>
<tr>
<td>DCP, 2 weeks</td>
<td>-0.891</td>
<td>-0.758</td>
<td>-0.873</td>
<td>-0.735</td>
</tr>
<tr>
<td>TCP, 1 week</td>
<td>-0.868</td>
<td>-0.983</td>
<td>-0.935</td>
<td>-0.857</td>
</tr>
<tr>
<td>TCP, 2 weeks</td>
<td>-0.973</td>
<td>-0.907</td>
<td>-0.972</td>
<td>-0.955</td>
</tr>
<tr>
<td>TCP, 3 weeks</td>
<td>-0.781</td>
<td>-0.634</td>
<td>-0.607</td>
<td>-0.612</td>
</tr>
<tr>
<td>PCP, 1 week</td>
<td>-0.745</td>
<td>-0.743</td>
<td>-0.804</td>
<td>-0.735</td>
</tr>
<tr>
<td>PCP, 2 weeks</td>
<td>-0.713</td>
<td>-0.622</td>
<td>-0.712</td>
<td>-0.610</td>
</tr>
<tr>
<td>PCP, 3 weeks</td>
<td>-0.566</td>
<td>-0.604</td>
<td>-0.432</td>
<td>-0.561</td>
</tr>
</tbody>
</table>
Table 2-3. Correlation coefficients for unrecoverable mass.

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Root Surface Area Index</th>
<th>Root Mass</th>
<th>Shoot Mass</th>
<th>Transpired Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCP, 1 week</td>
<td>0.942</td>
<td>0.772</td>
<td>0.842</td>
<td>0.751</td>
</tr>
<tr>
<td>DCP, 2 weeks</td>
<td>0.883</td>
<td>0.757</td>
<td>0.873</td>
<td>0.710</td>
</tr>
<tr>
<td>TCP, 1 week</td>
<td>0.798</td>
<td>0.947</td>
<td>0.897</td>
<td>0.831</td>
</tr>
<tr>
<td>TCP, 2 weeks</td>
<td>0.967</td>
<td>0.901</td>
<td>0.971</td>
<td>0.952</td>
</tr>
<tr>
<td>TCP, 3 weeks</td>
<td>0.781</td>
<td>0.636</td>
<td>0.610</td>
<td>0.618</td>
</tr>
<tr>
<td>PCP, 1 week</td>
<td>0.382</td>
<td>0.373</td>
<td>0.384</td>
<td>0.287</td>
</tr>
<tr>
<td>PCP, 2 weeks</td>
<td>0.661</td>
<td>0.547</td>
<td>0.647</td>
<td>0.571</td>
</tr>
<tr>
<td>PCP, 3 weeks</td>
<td>0.545</td>
<td>0.586</td>
<td>0.390</td>
<td>0.582</td>
</tr>
</tbody>
</table>

Some studies have suggested that transpiration is the dominant mechanism by which a compound is removed from the soil solution by a plant (Briggs, et al., 1982; Burken and Schnoor, 1997). In this experiment, a general pattern of increasing chlorophenol removal from solution with increasing transpired volume was observed. However, as quantified by correlation coefficients, the transpired volume tended to yield the weakest correlations with chlorophenol distribution.

In addition to the weak correlation between chlorophenol distribution and transpiration, the volume of water removed by the grass was not commensurate with the mass of chlorophenol removed. Several samples were characterized by minimal water losses but substantial chlorophenol removals. For example, one DCP sample exhibited a 16.3% mass removal with no measurable water loss (Figure 2-7a). For the PCP study, one sample from each incubation time was characterized by zero water loss, while between 63.5% and 29.4% of the PCP had been removed from these solutions by the grass (Figure 2-7b). Even in solutions characterized by measurable water loss, the percent mass remaining was consistently and substantially less than the percent volume of water remaining (Figures 2-7a and 2-7b). In addition, at the same transpired volume, different contact times were associated with different fractions remaining in solution. These results suggest that processes other than transpiration caused the removal of the chlorophenols from solution.
If translocation were the primary mechanism by which the plants rendered the chlorophenols unextractable, then the non-extractable fraction should be similar to the fraction of water transpired. However, it is clear that transpiration is also not required for rendering the chlorophenols unrecoverable (Figures 2-8a and 2-8b). The PCP data (Figure 2-8b) also demonstrate that, for the same volume of transpired water, the unrecoverable fraction substantially increased with time. For example, at week one, a 4% water removal corresponded to an unrecoverable fraction of 28% to 38%. At week two, the same transpired volume was associated with 56% to 62% of the PCP being unrecoverable. Since the unrecoverable fraction was strongly affected by sample time and since this fraction substantially
exceeded the fraction transpired, other mechanisms besides translocation were important in the unrecoverability process.

Figure 2-8. Comparison of the mass fraction of unrecoverable DCP and PCP to the fractional volume of transpired water.

To reflect the ability of the grasses to remove each chlorophenol from the bulk solution in an unrecoverable manner, a concentration factor was calculated:

\[
\text{Concentration Factor} = \left( \frac{\text{mg unrecoverable/L water transpired}}{\text{initial mg/L bulk solution}} \right)
\]
The resulting number is the amount by which the initial concentration would be multiplied in order to obtain the concentration in the transpiration stream if transpiration constituted the sole means by which the plant rendered the chlorophenol unrecoverable. This factor is similar in concept to the transpiration stream concentration factor (TSCF) described by Briggs et al. (1982) and used by Burken and Schnoor (1998). The two factors differ in that the TSCF of Burken and Schnoor (1998) considers only that portion of the contaminant translocated away from the roots while the concentration factor of this paper encompasses, in addition to the translocated mass, the mass rendered unrecoverable by other mechanisms, such as metabolic transformation and irreversible sorption.

As expected from the summarized data, all of the whole plant concentration factors were greater than one. During the first two weeks, the TSCF for TCP ranged from 1.39 and 7.19, while those for PCP ranged from 4.10 to 23.5. In general, for similar root surface area indices, the most nonpolar compound (PCP) yielded the greatest concentration factors.

The partitioning of a nonpolar, organic compound out of the aqueous phase onto organic matter is well documented (Schwarzenbach, et al., 1993). To assess the role of sorption in the observed results, the removal of each chlorophenol from solution was plotted as a Langmuir isotherm (Figures 2-9 and 2-10). The chlorophenol mass used in the solid concentration term was the sum of the extractable and unrecoverable fractions. At first, the solid concentration in the 1/q term was calculated on a per root mass basis. This approach yielded plots with a high degree of scatter (Figure 2-9). Recalculation of the solid concentration by replacing the root mass with the root surface area index resulted in approximately linear isotherms for the three chlorophenols at the various sample times (Figure 2-10). Although only the DCP isotherms are shown, isotherms for TCP and PCP followed the same pattern. Thus, the removal of the chlorophenols from solution could be modeled well as an adsorption process in which the root surface area index, not the root mass, was the dominant variable.
Mechanisms

Based on the data collected, a two stage process is proposed to describe the loss of chlorophenol from solution in the presence of rye grass. The first stage is removal from solution, and the second stage is unrecoverability of the removed...
contaminant. The different kinetics observed for removal and extractability support the separation of the plant/chlorophenol interactions into two stages.

The first stage of uptake by the grass is the movement of the chlorophenol out of solution by sorption with the roots. The movement of a hydrophobic compound out of an aqueous solution onto or into a solid organic phase has been well-established in the literature (Schwarzenbach, et al., 1993). The occurrence of this process is supported by the Langmuir isotherm results. The better fit achieved by basing the calculations on root surface area index suggests that adsorption contributed more to chlorophenol removal than absorption.

During the second stage, which actually occurred simultaneously with the partitioning but at a different rate, the grass interacted with the chlorinated phenol through one or more mechanisms that caused the compound to no longer be reversibly sorbed to the root surface. The literature has identified several potential mechanisms by which this process of rendering the compound unrecoverable can occur: diffusion into cells followed by ionization and inability to diffuse out (Schmidt, et al., 1994); translocation (Briggs, et al., 1982; Burken and Schnoor, 1998); and metabolism within the root and shoot tissues (Hatzios, 1988; Burken and Schnoor, 1997). The coupling of a chlorophenol to lignin by peroxidase is a potential transformation reaction that could occur within the roots and on the root surface (Barz and Koster, 1981). Regardless of the specific combination of mechanisms that occurred, the reduction in extractable fraction over time indicates that this process by which the chlorophenol could not be recovered from either the root surface or plant tissue continued throughout the study.

The continuous removal of the chlorophenols from solution suggests that partitioning equilibrium was not achieved until the chlorophenols were completely removed. In the sorption study using radiolabeled TCP and severed grass roots, no further sorption occurred between days 3 and 11, suggesting that, with roots alone, the sorption process achieved equilibrium in a couple of days. However, in the presence of live grass, chlorophenol removal from solution continued throughout the duration of the study (Figure 2-2).
In samples containing the same type of organic matter and same aqueous solution characteristics (such as activity coefficient), the ratio of the solid concentration and the aqueous concentration is constant (Schwarzenbach, et al., 1993). By metabolically transforming the chlorophenol (including such reactions as oxidative coupling of the compound to the root surface or reactions within the plant), translocating the compound away from the roots, or isolating an ionizable compound inside a cell membrane, the grass reduced the solid concentration at the root surface. To maintain the constant partition coefficient, more chlorophenol moved out of solution. Thus, the various interactions between the plant and the chlorophenol generated disequilibrium between the root surface and the aqueous phase that resulted in continued removal of the chlorophenol from solution.

The three chlorophenols yielded different rates of removal and unrecoverability, indicating that the molecular structure of the compound was a factor in both of those processes. Partitioning onto the root surface and movement within the plant would be affected by both hydrophobicity and dissociation constant (Schwarzenbach, et al., 1993; Briggs, et al., 1982; Burken and Schnoor, 1998). Although PCP was quickly removed from solution, on a per root surface area basis, this compound was the most slowly rendered unrecoverable. Once adsorbed, DCP and TCP, the more polar compounds, were more quickly rendered unrecoverable. The variety of possible mechanisms that result in unrecoverability, in particular the potential biochemical pathways, are likely affected in different ways by the contaminant structure. Before reliable predictions of preferential uptake of one compound over another can be made, it is necessary to conduct further research on the different mechanisms that result in the contaminant being rendered unrecoverable (in a practical sense, that result in the contaminant being isolated from the external media, such as groundwater and soil, by the plant).

**CONCLUSION**

To promote the removal of a compound from solution, maximization of the interface between the soil matrix and the roots should be considered when
selecting which plant species to use at a site. A plant characterized by a fibrous root network would provide a greater surface area for adsorption than a species with a tap root. Clearly, the species selection should also ensure that the roots can reach the zone of contamination. Although translocation is important as one possible mechanism for renewing the partitioning surface to allow further contaminant removal, the first step in direct phytoremediation is transferring the contaminant from the soil solution onto and into the root epiderm.

The fact that other mechanisms besides transpiration affect contaminant removal suggests that plants need not be actively growing to yield beneficial effects. Even during dormancy, sorption to roots would reduce the contaminant concentration in the surrounding solution. For ionizable compounds, diffusion into a cell and ionization would immobilize a fraction of the contaminant without requiring active plant growth. Whether or not enzymatic transformation would occur during dormancy requires exploration.

REFERENCES


CHAPTER 3: UPTAKE OF CHLOROPHENOLS BY RYE GRASS: ROLE OF PHYSICO-CHEMICAL PROCESSES

Cynthia E. Crane and John T. Novak

ABSTRACT

Rye grass was grown in the presence of 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP). The grass removed substantial amounts of TCP and PCP from solution. The majority of the removed contaminant could not be recovered by ethanol extraction. This unrecoverable fraction is considered to be “internalized” by the plant. The mass fractions removed and internalized were not commensurate with the fraction of water transpired, indicating that other mechanisms besides translocation were important in plant-contaminant interactions. The relative root surface area was correlated with both chlorophenol removal and internalization, suggesting that sorption of the contaminants onto the roots from solution was an important factor in plant-contaminant interactions.

INTRODUCTION

The use of plants to enhance in situ remediation appears to be a promising technology. Researchers have demonstrated that some plant species are able to directly take up organic contaminants from soil and transform them (Burken and Schnoor, 1997). Past research has identified pathways by which organic compounds enter the root endoderm from the surrounding solution and has explored the effect of contaminant characteristics on plant uptake (Briggs, et al. 1982). In terms of maximizing the rate and extent of remediation, there are many issues that require further research (Cunningham, et al., 1996). One such issue is how to promote the transfer of the contaminant from the surrounding soil solution into the roots. The goal of this research was to study the mechanisms for removal of contaminants from solution by plants.
MATERIALS AND METHODS

In order to isolate the interactions between the plants and the test compounds, all experiments were conducted in a hydroponic system. The experimental compounds were 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP), and the experimental plant was rye grass. Seeds were germinated in jars filled with semisolid bactoagar (6 grams bactoagar/liter nutrient solution). After germination, the seedlings were grown under cool, white lights with a 14 hour photoperiod for three weeks prior to experimental use.

Fifty mL of a chlorophenol/nutrient solution were pipetted into 70 mL, straight-sided glass jars. The initial concentrations were 0.0242 mM PCP and 0.0377 mM TCP. Seedlings were removed from agar, rinsed and then anchored into place in the jars using cotton, parafilm and tape. The number of plants in each jar was varied in order to determine which variables, such as root mass and transpired volume, were most closely related to compound removal. Each sampling episode consisted of jars containing from ten to forty seedlings and three jars containing no plants to monitor system loss.

Replicates were sacrificed at week one and week three. The shoots were clipped from the roots and weighed. The roots were weighed, placed in screwtop vials and extracted with ethanol for 24 hours. After extraction, the root surface area index was determined. The chlorophenol content of the root extract was analyzed on a Hewlett Packard 5890A Gas Chromatograph fitted with an electron capture detector. The aqueous samples were extracted with hexane and the extracts were analyzed by gas chromatography.

The relative surface area of the roots was measured by adsorption of rhodamine red (Sorenson and Wakeman, 1996). The roots were placed in 125 mL Erlenmeyer flasks containing 30 mL of a 2 µM rhodamine red solution at pH 4.9. The flasks were agitated for 18 hours on a shaker table and then the solution was centrifuged at 10,000 rpm for 10 minutes. The absorbance of the centrate was measured on a spectrophotometer at a wavelength of 550 nm. The adsorbed mass, expressed as umoles, is referred to throughout the paper as the root surface area index and reflects the relative adsorptive capacity of the different samples.
RESULTS AND DISCUSSION

Control losses were minimal during the first week: 6.3% of the PCP and 11.6% of the TCP. By three weeks, the control losses were more substantial: 38.5% for PCP and 50.5% for TCP. To normalize the sample values, mass fractions were calculated relative to the mass remaining in the corresponding controls.

Within one week, the amount of TCP and PCP in solutions containing grass decreased substantially (Figure 3-1). Depending on the amount of roots present, up to 59% of the PCP and 75% of the TCP were removed. The mass remaining in solution after one week was correlated with root mass (correlation coefficients of -0.684 for PCP and -0.870 for TCP) and root surface area index (correlation coefficients of -0.834 for PCP and -0.969 for TCP). After three weeks, up to 95% of the TCP and 69% of the PCP had been removed from solution. The samples with the highest root surface area indices contained as little as 0.021 mg TCP/L (0.0011 mM, Figure 3-1). After 21 days, the mass of PCP remaining in solution yielded correlation coefficients of -0.727 with respect to root mass and -0.607 with respect to root surface area index. For TCP, the correlation coefficients were -0.905 for root mass and -0.897 for root surface area index. At both sample times, TCP was preferentially removed from solution.

![Figure 3-1. Relationship between root surface area and aqueous concentration.](image-url)
Transpiration is thought to be an important mechanism by which plants remove a compound from solution. While water removal was correlated with the removal of both TCP and PCP (correlation coefficients ranging from 0.615 to 0.854), the mass fraction removed was not commensurate with the volume fraction of water removed (Figure 3-2). For example, in one of the day 7 samples, no transpiration was measured while 38.1% of the TCP was removed from solution. In another day 7 sample, a TCP mass fraction removal of 74.5% coincided with a transpired fraction of 4.08%.

![Figure 3-2](image)

Figure 3-2. The TCP mass fraction removed was consistently greater than the volume fraction of water transpired.

Most of the TCP and PCP that had been removed from solution could not be recovered by extraction with ethanol. The chlorophenol mass extracted from the roots by ethanol was divided by the chlorophenol mass removed from solution to yield the extractable fraction. This fraction was very small for TCP, less than 3.52% at day 7 and less than 1.01% at day 21, indicating that very little of the TCP was desorbed. On the other hand, at 7 days, between 39.9% and 83.7% of the PCP removed from solution was recovered by ethanol extraction of the roots. This result suggests that the majority of the PCP either was reversibly associated with the root surface or had reversibly diffused into the epiderm. Between day 7
and day 21, the extractable PCP fraction decreased to less than 5.7%, indicating that the majority of the PCP had been rendered unrecoverable to the external system by the plant between day 7 and day 21.

In this paper, the amount of each compound that could not be recovered is referred to as “internalized”, a purely operational definition. This mass was calculated by subtracting the chlorophenol mass extracted from the roots and the mass of chlorophenol remaining in solution from the mass present in the controls. The difference in the TCP and PCP extractable fractions at day 7 and the substantial decrease in the PCP extractable fraction between day 7 and day 21 indicate that removal and internalization follow different kinetics.

Particularly at day 7, a greater mass of TCP was internalized on a per root surface area basis as compared to PCP (Figure 3-3). At 7 days, as much as 32.7% of the PCP and 72.1% of the TCP was internalized. The mass of internalized PCP was not correlated with either root mass or root surface area index, while the mass of internalized TCP had correlation coefficients of 0.858 with respect to root mass and 0.970 with respect to root surface area index. After three weeks, up to 68.2% of the PCP and 95.2% of the TCP was internalized. The mass fraction of internalized PCP was correlated with root mass (correlation coefficient of 0.733) and root surface area index (coefficient of 0.615). The internalized TCP fraction exhibited strong relationships with both root mass (correlation coefficient of 0.906) and root surface area index (correlation coefficient of 0.897). Thus, TCP was preferentially internalized over PCP, and the internalization of TCP was more strongly related to the amount of roots present than was the internalization of PCP.

One of the mechanisms by which plants may internalize contaminants is through translocation. As shown in Figure 3-4, the fraction internalized was not commensurate with the fraction transpired. For example, one of the day 21 samples did not exhibit measurable transpiration. In this sample, the grass had internalized 37.7% of the PCP and 36.0% of the TCP. Thus, although translocation appears to play a role in the internalization process, it is not the only mechanism.
Figure 3-3. The relative propensity of TCP and PCP to be internalized by the grass, day 7.

Figure 3-4. The internalized fraction of TCP or PCP at day 7 was substantially greater than the volume fraction removed by transpiration.

From the data, it is clear that TCP was preferentially removed and preferentially internalized over PCP. In addition, the much greater removal and internalization of both chlorophenols indicates that the compounds concentrate either in the vicinity of the roots or on the roots. To assess the extent of this concentration effect, a concentration factor was calculated in the following manner:
Concentration Factor = \( \frac{\text{mg internalized/L transpired}}{\text{initial mg/L bulk solution}} \)

The resulting number is the amount by which the initial concentration would be multiplied in order to obtain the concentration in the transpiration stream if transpiration constituted the sole means by which the plant internalized the chlorophenol. This factor is similar in concept to the transpiration stream concentration factor (TSCF) described by Burken and Schnoor (1998).

Consistent with the preferential removal and internalization of TCP over PCP, the concentration factors were consistently higher for TCP than PCP. At day 7, the concentration factors ranged from 9.16 to 23.8 for PCP and from 11.4 to 25.4 for TCP. At day 21, these values were between 2.56 and 18.4 for PCP and between 4.47 and 18.3 for TCP.

These data suggest that, during removal from solution, the chlorophenol accumulates on or in the vicinity of the roots. Within the aqueous system, roots provide an organic surface with which a hydrophobic compound may associate. Thus, it is likely that the first step of a contaminant being directly remediated by a plant is the adsorption of the contaminant onto the root tissue and absorption into the epiderm. Based strictly on polarity, PCP should exhibit a greater affinity with the roots than TCP. However, because PCP’s pKa is 4.74 while TCP’s is 5.99 (Westall, et al., 1985; solution pH was 5), a greater fraction of PCP would be in the phenolate form as compared to TCP. Ionization reduces the force driving the compound to move out of an aqueous matrix (Schwarzenbach, et al., 1993).

Another explanation for the preferential removal of TCP over PCP lies in the preferential internalization of TCP. Once equilibrium between the chlorophenol in solution and the chlorophenol on the roots is reached, further sorption should not occur unless the aqueous concentration is augmented or the solid concentration is reduced. The proposed internalization mechanisms, by moving or transforming the compound, would effectively reduce the chlorophenol concentration in the parts of the root exposed to the aqueous system. Thus,
internalization would allow sorption, or removal, to continue. The slower rate at which PCP was removed was due to its slower internalization. Briggs, et al. (1982) hypothesized that very nonpolar compounds, although readily able to diffuse across membranes, could not redissolve into the symplast. Thus, it is likely that the TCP was more readily moved into the endoderm than the PCP, increasing the rate of internalization and thus promoting removal.

CONCLUSION

This study demonstrates that plants have potential to directly promote in situ remediation. The results indicate that, as the first stage in direct plant-contaminant interactions, physico-chemical interactions are extremely important. Since it is not necessary for a plant to transpire in order for substantial amounts of an organic compound to be removed, beneficial effects may be achieved even during dormant seasons. The data also indicate that root surface area is of great importance in contaminant removal. Thus, in species selection for design of an in situ remediation system, should consider the use of plants characterized by high root surface areas, such as grasses with a fine root network capable of maximizing contact between the plant and the contaminated soil.

REFERENCES


CHAPTER 4: EFFECT OF RYE GRASS ROOTS ON THE SOLVENT PARTITIONING OF CHLORINATED PHENOLS

ABSTRACT

Three chlorinated phenols, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol, were placed separately into aqueous solution which were subsequently adjusted to either pH 4.9 or pH 6.3. Different masses of rye grass roots were added to 20 mL of the solution and 10 mL of hexane were pipetted onto the water surface. After 24 hours of quiescent contact, the solvent layer was removed, the aqueous phase was analyzed and the root surface area index was determined. The chlorophenol mass that partitioned into the hexane was inversely correlated with root mass and root surface area index. Partition coefficients calculated with respect to the hexane phase were also inversely correlated with root mass and root surface area index. A similar partitioning experiment was conducted using PCP placed in a solution containing only the dissolved organic material released by roots. The resulting partition coefficients decreased with increasing organic carbon concentration. It appears that the organic compounds released into solution by the roots affected the movement of the chlorophenol into the hexane phase. The presence of roots simultaneously promoted retention of the chlorophenols in the aqueous phase and provided a sorption site.

INTRODUCTION

The use of plants to enhance in situ remediation of soils contaminated by organic pollutants is a newly emerging technology. From pesticide research, the means by which plants take up, translocate and metabolize organic chemicals have been identified. However, since pesticide research is not conducted with the intent of maximizing the removal of organic compounds from the soil or achieving remediation goals in the shortest period of time, there remain several issues which require further research (Cunningham, et al., 1996). One question concerns the potential extent of the role that physico-chemical interactions between roots and contaminants may play during site remediation.
The partitioning of a hydrophobic solute from the aqueous phase into an adjacent organic phase results from the incompatibility of the nonpolar molecule with the hydrogen-bonded aqueous matrix (Schwarzenbach, et al., 1993). This movement out of the aqueous phase can be achieved through interactions with organic surfaces, diffusion into an organic matrix, or dissolution into an adjacent organic liquid. Within soils, the incompatibility of a hydrophobic compound with water results in the movement of a substantial fraction of the compound onto or into soil organic matter. The extent of association with the soil organic matter depends on the hydrophobicity of the solute, often quantified by the octanol-water partition coefficient ($K_{ow}$), and the concentration of organic matter in the soil matrix (Schwarzenbach, et al., 1993). Many organic contaminants, such as phenols, ionize in solution. Since the ionic form is more compatible with the aqueous phase than the nonionic form, the degree of ionization, and thus the solution pH, affects the extent of partitioning (Schwarzenbach, et al., 1993).

The association of hydrophobic compounds with soil organic matter affects the fate and remediation of these contaminants in the subsurface system. Phenols and anilines may be incorporated into humic material by oxidative coupling, an enzymatic reaction that irreversibly binds and transforms the contaminants (Berry and Boyd, 1984). Adsorbed contaminants may be rendered unavailable with respect to microbial metabolism (Ogram, et al, 1985; Burgos, et al., 1996). The association with soil organic matter reduces the extractability of contaminants (Crane and Novak, 1997), potentially interfering with chemical analyses and the effectiveness of pump-and-treat systems. Since association with organic matter makes site remediation more difficult, reductions in the degree of partitioning would facilitate in situ remediation. The basic intent was to determine the adsorptive competitiveness for chlorophenol of roots as compared to a solvent to better describe the mechanisms by which plants can accumulate organic pollutants in their roots. These experiments considered the interactions between rye grass, a species that is easy to grow and maintain, and chlorinated phenols, chemicals that are widespread soil contaminants.
METHODS AND MATERIALS

The experimental solutes were 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP). These structurally similar compounds encompass a range of polarity (log K_{ow} from 2.75 to 5.01) and dissociation constant (pK_{a} from 4.74 to 7.85) (Westall, et al., 1985; Table 4-1). The most hydrophobic compound should yield the greatest extent of movement out of the aqueous phase. However, the most hydrophobic experimental compound (PCP) is characterized by the lowest pK_{a}. From a hydrophobicity perspective, the observed degree of partitioning should be PCP > TCP > DCP. Since the dissociation of the chlorophenols under mildly acidic conditions is greatest for the most hydrophobic compound, the most hydrophobic compound would have the greatest ionized fraction in solution. The ionized form has a lower tendency to partition than the nonionized form. Thus, the relative extent of partitioning for the test compounds might not follow the pattern predicted by hydrophobicity alone.

Table 4-1. Properties of the Experimental Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Weight</th>
<th>Solubility (mg/L)</th>
<th>pKa</th>
<th>log K_{ow}</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCP</td>
<td>164</td>
<td></td>
<td>7.85</td>
<td>2.75</td>
</tr>
<tr>
<td>TCP</td>
<td>197</td>
<td>800</td>
<td>6.01</td>
<td>3.38</td>
</tr>
<tr>
<td>PCP</td>
<td>266.5</td>
<td>14</td>
<td>4.74</td>
<td>5.01</td>
</tr>
</tbody>
</table>

Rye grass seeds were germinated in semisolid bactoagar. After two to three weeks of growth, the grasses were removed from the agar and the roots were severed from the shoots. Different amounts of roots, ranging from less than 100 mg to 1200 mg, were weighed and placed into 40 mL, screwtop vials containing 20 mL of a chlorinated phenol/dilute nutrient solution adjusted to either pH 4.9 or pH 6.3. The three compounds were studied individually in solution. The initial concentrations for each chlorophenol were 0.0164 mM to 0.0180 mM for DCP and PCP, and 0.0233 mM for TCP. Ten mL of hexane were pipetted immediately onto the water surface. Each treatment had duplicate, rootless controls. The two
phases were not mixed together, but were simply allowed to contact under quiescent conditions. After 24 hours, the solvent layer was removed by pipette and analyzed for chlorophenol content on a Hewlett Packard 5890A Gas Chromatograph fitted with an electron capture detector, an autosampler and a Supelco DB5 column. The aqueous chlorophenol concentration was determined as described below. The root surface area index was measured using the method described in the following section.

Five mL of each aqueous sample was placed into an 11 mL screwtop vial and acidified to below pH 2 with 4 drops of 1 N sulfuric acid. For TCP, 3 mL of hexane were pipetted onto the aqueous phase, and the two phases were mixed by wrist action. The phases were allowed to separate and the hexane was removed from the aqueous sample with a Pasteur pipette and placed into another screwtop vial. This process was repeated twice. Finally, one mL of the hexane extract was placed into an autosampler vial and the remaining hexane volume was measured. For DCP and PCP, 3 mL of hexane was pipetted onto the aqueous phase. The two phases were not mixed together but, instead, were allowed to contact under quiescent conditions for 24 hours before removal of the hexane by Pasteur pipette. Next, one mL of the hexane extract was placed in an autosampler vial and the remaining hexane volume was measured. The extracts were analyzed on a Hewlett Packard 5890A Gas Chromatograph fitted with an electron capture detector, an autosampler and a Supelco DB5 column. The sample standard deviation for extraction and analysis was 12.1% for DCP (n = 30), 9.09% for TCP (n = 55), and 6.89% for PCP (n = 25).

During initial experiments, the aqueous phase turned brown, indicating that the severed roots released dissolved organic material. This hypothesis was confirmed by soaking roots in a nutrient solution overnight and measuring the subsequent total organic carbon content. An experiment was conducted to determine if this dissolved material affected partitioning. Different masses of roots were added to 25 mL of the nutrient solution at pH 4.9. After 24 hours, the roots and a five mL aqueous sample were removed from each vial. This five mL sample was analyzed for total organic carbon (TOC) content on a Horiba PIR-
2000. The remaining 20 mL were spiked with PCP to an initial concentration of 0.0236 mM, and 10 mL of hexane were pipetted onto the surface of the water. Partitioning was allowed to proceed for 24 hours, after which time the vials were sampled and analyzed as described above.

**Root Surface Area Index**

Measurement of the adsorption of rhodamine red by roots was used to determine relative surface areas of the samples (Sorenson and Wakeman, 1996). The roots were placed into 125 mL Erlenmeyer flasks containing 30 mL of a rhodamine red solution with a concentration of 2 µM and at a pH of 4.9. The flasks were agitated for 18 hours on a shaker table to achieve equilibrium prior to centrifugation at 10,000 rpm for 10 minutes. The absorbance of the centrate was measured on a spectrophotometer at a wavelength of 550 nm. The adsorbed mass, expressed as umoles, is referred to throughout the paper as the root surface area index and reflects the relative adsorptive capacity, and thereby the relative surface area, of the different samples.

**RESULTS**

The presence of roots in the aqueous phase reduced the movement of each chlorinated phenol into hexane as compared to the rootless controls. The concentration of each chlorophenol in the hexane layer was inversely related to both the root surface area index and the root mass (Tables 4-2 and 4-3). In samples containing the highest amount of roots, the concentration in hexane was reduced by as much as 60% to 75% compared to the rootless controls.

The inverse relationship between the chlorophenol mass fraction in the hexane layer and the root surface area index was approximately linear (Figures 4-1a, 4-2a and 4-3a). At pH 4.9, a greater fraction of PCP moved into the hexane than at pH 6.3 (Figure 4-1a). At the higher root surface area indices, the PCP hexane fractions from both pH treatments were similar, with approximately 25% of the PCP in the hexane phase. DCP exhibited similar hexane fractions for a given root surface area at both pH 4.9 and 6.3 (Figure 4-2a). While an increase in
pH from 4.9 to 6.3 yields relatively little change in the extent of ionization for DCP (pKa of 7.85), the same pH increase substantially increases the fraction of PCP in the phenolate form (from \( \approx 50\% \) to \(< 90\%\)). The differences in the influence of pH on DCP and PCP indicate, as expected, that the degree of ionization affects the partitioning into hexane in the presence of grass roots.

Table 4-2. Correlation coefficients with respect to root mass.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Hexane Fraction</th>
<th>Root-Associated Fraction</th>
<th>Partition Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCP, pH 4.9</td>
<td>-0.737</td>
<td>0.677</td>
<td>-0.761</td>
</tr>
<tr>
<td>PCP, pH 4.9</td>
<td>-0.942</td>
<td>0.942</td>
<td>-0.873</td>
</tr>
<tr>
<td>DCP, pH 6.3</td>
<td>-0.888</td>
<td>0.962</td>
<td>-0.761</td>
</tr>
<tr>
<td>TCP, pH 6.3</td>
<td>-0.950</td>
<td>0.912</td>
<td>-0.768</td>
</tr>
<tr>
<td>PCP, pH 6.3</td>
<td>-0.907</td>
<td>0.857</td>
<td>-0.658</td>
</tr>
</tbody>
</table>

Table 4-3. Correlation coefficients with respect to root surface area index.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Hexane Fraction</th>
<th>Root-Associated Fraction</th>
<th>Partition Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCP, pH 4.9</td>
<td>-0.926</td>
<td>0.888</td>
<td>-0.905</td>
</tr>
<tr>
<td>PCP, pH 4.9</td>
<td>-0.961</td>
<td>0.909</td>
<td>-0.975</td>
</tr>
<tr>
<td>DCP, pH 6.3</td>
<td>-0.941</td>
<td>0.960</td>
<td>-0.868</td>
</tr>
<tr>
<td>TCP, pH 6.3</td>
<td>-0.960</td>
<td>0.938</td>
<td>-0.733</td>
</tr>
<tr>
<td>PCP, pH 6.3</td>
<td>-0.918</td>
<td>0.786</td>
<td>-0.759</td>
</tr>
</tbody>
</table>
Figure 4-1. Distribution of PCP among the three phases.
Figure 4-2. Distribution of DCP among the three phases.
Figure 4-3. Distribution of TCP among the three phases.
At pH 4.9, PCP was typically characterized by a greater mass fraction in the hexane phase than DCP (Figure 4-4). The fractions of DCP and PCP in hexane were similar when the root surface area index exceeded 0.04 umoles of adsorbed rhodamine red. Based solely on compound hydrophobicity, a greater fraction of PCP should be in the organic phase as compared to DCP. At pH 6.3, PCP was characterized by the lowest mass fraction in the hexane phase while TCP generally yielded the greatest fraction (Figure 4-5). The fact that, at pH 6.3, the most hydrophobic compound resulted in the least movement into the hexane phase indicates the relative importance of ionization in controlling partitioning into the organic liquid. At pH 6.3, greater than 90% of the PCP was ionized, as compared to approximately 50% of the TCP and less than 10% of the DCP. In general, the extractive capability of the hexane was limited by the presence of roots.

![Graph showing partitioning of PCP and DCP into hexane from pH 4.9 solution and in the presence of grass roots.](image)

Figure 4-4. Comparison of partitioning of PCP and DCP into hexane from pH 4.9 solution and in the presence of grass roots.

It was expected that the addition of roots, by increasing the amount of organic sorbent in the system, would decrease the aqueous concentration, and thus decrease the mass fraction remaining in the aqueous phase. On the contrary, the addition of low to moderate amounts of roots increased the aqueous fraction (Figures 4-1b, 4-2b and 4-3b). As the amount of roots in the system increased,
the aqueous fraction reached a maximum and then declined (Figures 4-1b, 4-2b and 4-3b). For example, at pH 6.3, the DCP aqueous concentration in the rootless controls was 0.00552 mM, resulting in 32.0% of the total DCP mass in the aqueous phase. In the presence of roots with a surface area index of 0.0138 µmoles rhodamine red, the aqueous concentration was 0.0087 mM DCP, corresponding to an aqueous mass fraction of 50.5%. In the sample with a root surface area index of 0.0430 µmoles rhodamine red, the greatest root surface area of this treatment, the aqueous concentration decreased to 0.00502 mM, or an aqueous mass fraction of 29.1%. For DCP, the pH 6.3 samples yielded the greatest increase in the aqueous fraction upon the addition of roots. On the other hand, for PCP, the addition of roots caused a relatively greater increase in the aqueous mass fraction at pH 4.9 than pH 6.3, even though the aqueous fraction at pH 4.9 was less than that at pH 6.3 (Figure 4-1b).

As noted above, the DCP mass fraction in the hexane phase was similar at pH 4.9 and pH 6.3 (Figure 4-2a). However, in samples characterized by root surface area indices between 0.01 and 0.03 µmoles rhodamine red, the DCP aqueous mass fraction at pH 6.3 was substantially greater than at pH 4.9 (Figure 4-5).
For PCP, in samples containing roots with a surface area index less than 0.03 µmoles rhodamine red, the aqueous mass fraction tended to be lower at pH 4.9 than at pH 6.3 (Figure 4-1b). Above 0.03 µmoles of adsorbed rhodamine red, the pH 4.9 and 6.3 samples yielded similar aqueous mass fractions for both compounds.

In the absence of roots, at pH 4.9, the PCP aqueous fraction was less than that of DCP (Figure 4-6). In the root-containing samples, the aqueous fractions of DCP and PCP were similar (Figure 4-6). A similar pattern was observed for DCP and PCP at pH 6.3, except that DCP had the lesser concentration in the rootless controls (Figure 4-7). Thus, the addition of roots appeared to overcome the effect of contaminant hydrophobicity on the tendency of the compound to remain in the aqueous phase.

![Figure 4-6. Comparison of DCP and PCP aqueous fractions, pH 4.9](image)

In addition to promoting the retention of the chlorophenols by the aqueous phase, the roots acted as a sorbent. Based on mass balance calculations, up to 50% of each chlorinated phenol became associated with the roots. This root-associated fraction was strongly correlated with both root mass and root surface area index (Tables 4-2 and 4-3). Contrary to what one might expect from the relative extracting strengths of roots and hexane, a relatively small volume of roots substantially reduced movement into the hexane.
To assess the effect of roots on the movement into the hexane phase, hexane partition coefficients ($K_p$) were calculated by dividing the hexane concentration by the aqueous concentration. In the literature, it is assumed that organic carbon partition coefficients in soil are constant for a given compound (Schwarzenbach, et al., 1993). However, the presence of roots affected the $K_p$, causing it to decrease with increasing root surface area index (Figures 4-8 to 4-11). The $K_p$'s were inversely correlated with respect to both root mass and to root surface area index (Tables 4-2 and 4-3).

For PCP, pH appeared to exert substantial influence over the $K_p$ in the absence of roots and in the presence of relatively low surface area indices (Figure 4-8). As the root surface area index increased, the PCP $K_p$’s for pH 4.9 and pH 6.3 became similar. While the linear relationship between $K_p$ and root surface area index is relatively strong for PCP at pH 4.9, this relationship is weaker at pH 6.3. For DCP, the $K_p$’s for pH 4.9 were lower than those at pH 6.3 in the absence of roots and in the presence of low amounts of roots (Figure 4-9). Based on the similarities in the hexane fraction at both pH’s, one might have expected the $K_p$’s to be similar also. However, in this range of root surface area indices, the aqueous concentration at pH 4.9 was higher than that for pH 6.3 (Figure 4-2b).
is this effect of the roots on the aqueous concentration that resulted in the disparity between the pH 4.9 and pH 6.3 $K_p$’s at low root surface area indices. Above a root surface area index of 0.03 umoles rhodamine red adsorbed, the aqueous DCP concentration was similar for both pH’s. At this same point, the $K_p$’s for DCP at both pH’s became similar.

Figure 4-8. Decrease in the hexane partition coefficient for PCP with increasing root surface area.

Figure 4-9. Decrease in the hexane partition coefficient for DCP with increasing root surface area.
As would be expected from the relative contaminant hydrophobicity, the PCP $K_p$’s were greater than DCP $K_p$’s in the absence of roots and presence of low amounts of roots at pH 4.9 (Figure 4-10). However, as the amount of roots increased, the $K_p$’s for both chlorophenols became similar. At pH 6.3, PCP tended to yield the lowest $K_p$’s while TCP was characterized by the highest $K_p$’s (Figure 4-11). These latter results are scattered and the differences are not substantial.

Figure 4-10. With no roots and small to moderate amounts of roots, the PCP partition coefficients were greater than those for DCP.

Figure 4-11. Comparison of the partition coefficients for the three chlorophenols in the presence of roots at pH 6.3.
In summary, the roots substantially reduced the mass of each chlorophenol that partitioned into the hexane layer. The presence of roots influenced the amount of each compound that remained in the aqueous phase, raising the concentration above that found in the rootless controls at low root concentrations. For PCP, the presence of large amounts of roots tended to reduce differences in partitioning due to solution pH. At pH 4.9, the presence of large amounts of roots tended to reduce differences in partitioning due to solute hydrophobicity. In the presence of roots, the fraction remaining in the aqueous phase appeared to be more strongly affected by pH than compound hydrophobicity. In samples containing large amounts of roots, pH did not appear to influence the aqueous concentration. Finally, the partition coefficient was not constant: it was inversely related to both root mass and root surface area index, with the latter yielding the stronger relationship.

**Partitioning in Presence of Root-Derived TOC and Absence of Roots**

The severed roots released substantial amounts of dissolved organic material into solution. The specific amount released depended directly on the root mass initially present in the vials in an approximately linear manner (Figure 4-12). Thus, the roots added cosolutes to the aqueous phase. If present in sufficient quantities, cosolutes may affect the physico-chemical nature of the aqueous phase itself (Schwarzenbach, et al., 1993). By affecting the physico-chemical nature of the aqueous phase, the cosolutes would also affect partitioning into an adjacent organic phase. This reasoning is directly supported by the experimental results. The fraction of PCP that remained dissolved in the aqueous phase increased with increasing dissolved TOC, ranging from an average of 16.5% for the pure nutrient solution to 37.1% at 275 mg/L dissolved TOC. Thus the partition coefficient was inversely related to the dissolved TOC, decreasing from a control average of 10.9 to 3.40 at 275 mg/L dissolved TOC. These observations could have occurred only if the aqueous phase had been altered to be more compatible with the hydrophobic PCP.
The aqueous fractions of PCP obtained in the presence of roots and in the presence of only root-derived organic carbon were compared on a root mass basis (i.e., the root mass from which the dissolved organic carbon for the rootless samples had been obtained, Figure 4-13). At the low root masses, the samples containing actual root tissue yielded higher aqueous fractions than the ones with only the dissolved material. As root mass increased (dissolved TOC increased), the rootless aqueous fraction increased to exceed the aqueous fractions observed in the root-containing samples. These results have two implications. The first is that the aqueous concentration enhancement was primarily due to dissolved organic carbon released into solution by the roots. The second implication is that observed decreases in the aqueous fraction in the presence of actual root tissue suggests that removal of the chlorophenol by sorption was overwhelming any tendency for the dissolved organic carbon to increase the aqueous concentration.

![Graph showing dissolved TOC concentration vs. root mass](image)

Figure 4-12. The dissolved TOC concentration obtained from soaking rye grass roots of different masses in the pH 4.9 solution for 24 hours.
Figure 4-13. Fraction of PCP remaining in a pH 4.9 solution adjacent to a hexane layer. In one treatment, rye grass roots were present. In the other, only the dissolved organic matter released from the specified root mass was present.

**DISCUSSION**

Based on the results and the literature, two mechanisms appear to simultaneously govern the interactions between the experimental compounds and the roots: aqueous interactions among cosolutes; and sorption reactions.

The three test compounds are ionizable, hydrophobic compounds characterized by log $K_{ow}$'s of 2.75, 3.38 and 5.01 for DCP, TCP and PCP, respectively (Westall, et al., 1985). Water-organic liquid partition coefficients and aqueous solubility are functions of the compound's aqueous activity coefficient: the higher the aqueous activity coefficient, the more the compound partitions out of water (higher ln $K_{ow}$) and the lower the aqueous solubility (Schwarzenbach, et al., 1993). The aqueous activity coefficient of an organic compound may be affected by the presence of other organic compounds in solution. Research by Chiou indicated that the aqueous solubility of hydrophobic organic compounds was higher in octanol-saturated water than in pure water (as cited in Schwarzenbach, et al., 1993, p. 134). According to the authors, the
cosolute octanol decreased the aqueous activity coefficients of the test compounds, rendering the solvent-saturated aqueous solubility greater than that possible in a pure water system. Chiou (1986) also observed a solubility increase in aqueous solutions to which natural organic matter had been added.

The effect of activity coefficient on liquid-liquid partitioning can be readily noted once the partition coefficient is expressed in thermodynamic terms (Schwarzenbach, et al., 1993, p. 127):

$$\ln K_{sw} = \ln \gamma_w - \ln \gamma_s + \ln\left(\frac{V_w}{V_s}\right) = -\Delta G_{sw}^{\circ}/RT + \text{constant}$$

where $K_{sw}$ is the solvent-water partition coefficient, $\gamma_w$ and $\gamma_s$ are the compound activity coefficients in water and the solvent phase respectively, and $V_w$ and $V_s$ are the molar water and solvent volumes, respectively. With constant temperature and volumes, as the cosolute decreases the $\gamma_w$ term, the free energy of reaction becomes less negative, or less favorable with respect to movement of the compound out of water. For these experiments, to emphasize the root effect, the $\gamma_w$ should be referred to as $\gamma_{w\text{rootmix}}$, since the roots release organic material into solution. The $\gamma$'s for the chlorophenols with respect to both hexane and ethyl acetate should be relatively small, along the lines of $10^0$ to $10^1$, while the $\gamma_s$'s should be in the range of $10^3$ or higher (order of magnitude estimates based on activity coefficients and log $K_{ow}$ for benzene, Schwarzenbach, et al., 1993). The addition of organic cosolutes should exert a greater effect on the $\gamma_{\text{rootmix}}$ than on the $\gamma_s$.

The underlying cause for the partitioning out of water is the incompatibility of the nonpolar molecule with the hydrogen-bonded, water matrix. The activity coefficient represents the various energy terms that comprise the free energy of the dissolution of the compound into water (Schwarzenbach, et al., 1993). If present in sufficient quantities, organic cosolutes may interact with the other hydrophobic molecules, overlapping the shells of hydration and thus reducing the energy necessary to dissolve the compound of interest (Schwarzenbach, et al., 1993). With respect to this research, the lowest masses of
roots may not have added sufficient cosolutes to the system to interact with the chlorophenols and thereby lower the energy of dissolution. As the greater root masses added higher concentrations of organic cosolutes to the water, the cosolute effect on the activity coefficient became evident. That the observed enhanced aqueous solubility was due to the decrease in activity coefficient by the dissolution of root organic matter is demonstrated by the increased aqueous fraction in the presence of only the root-derived soluble organic material.

One possibility that must be noted is that the large amounts of dissolved TOC released by the roots is an experimental artifact from the severing of the roots from the shoots. However, plants do release organic compounds into soil solution during growth (Tate III, et al., 1991). Upon tissue sloughing or plant death, the decay process will result in the dissolution of some of the root organic content into the soil solution. On the other hand, because soils typically contain some amount of organic matter, soil solutions do contain some dissolved organic carbon. Thus, the amount of TOC dissolved in a soil solution on a planted site will depend on a number of variables, including but not limited to the number of plants, the plant species and the soil organic content.

For these experiments, the addition of roots not only provided cosolutes, but it also furnished solid tissue upon and within which sorption reactions could take place. Since compounds could diffuse throughout the root tissue, both adsorption and absorption may have occurred. By simultaneously releasing cosolutes that promote dissolution in the aqueous phase and providing a sorbate for removal by sorption, whether the roots would increase or decrease the aqueous concentration would depend on the relative strength of these two mechanisms. Since a soil solution is likely to have high amounts of cosolutes in the absence of roots, it is unlikely that the roots would exert an appreciable influence on the aqueous activity coefficient. In such a situation, the sorption mechanism should dominate, allowing for simultaneous decrease in the aqueous concentration and in the amount of contaminant that would sorb to the soil organic matter.

It should be noted that reverse partitioning studies were conducted in which the hexane layer was initially spiked with chlorophenols and the movement
of the compounds into the underlying aqueous phase was measured after 24 hours. No measurable movement of the chlorophenols out of the hexane occurred. Thermodynamically, some dissolution into the water should have taken place. It is likely that the kinetics of dissolution were slower than the 24 hours allowed by the study. To determine the ability of roots to promote desorption of contaminants adsorbed to soil organic matter, studies should be conducted that take into account the slow kinetics of desorption.

CONCLUSION

The data indicate that roots can substantially decrease the movement of chlorophenols into a second organic phase (hexane). If plants were present at a new spill site, their roots could reduce the amount of contaminants that adsorb to soil organic matter, thereby facilitating future remediation. Since soil is characterized by a high surface area, the degree to which roots may reduce sorption onto the soil phase requires study. Due to the purely physico-chemical nature of the interactions, a beneficial effect should occur even when the plants are dormant. Placement of plants near tanks, pipelines and other sites of potential contamination could minimize the obstacles to remediation of future contamination, especially if the plants translocate and transform the contaminants. By adsorbing compounds from contaminated groundwater, plant roots may form a screen to retard offsite contaminant transport. Due to the time necessary to establish a plant population with a sufficiently extensive root network to serve as a groundwater screen, it might be easier to construct a barrier/membrane system. However, the potential plant root effect could be exploited to reduce subsurface transport from nonpoint source pollution. Regardless of specific use, the plants should be selected to maximize the volume of soil exposed to the root surfaces. A species with fine, extensive roots should be the most effective at competing with soil organic matter for dissolved contaminants.
REFERENCES


CHAPTER 5: INFLUENCE OF RYE GRASS ROOTS ON THE SOLVENT PARTITIONING OF MIXED CHLORINATED PHENOLS

ABSTRACT

Three chlorinated phenols, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol, were combined in an aqueous solution at either pH 4.9 or pH 6.3. Different masses of rye grass roots were added to 20 mL of the solution and 10 mL of either hexane or ethyl acetate were pipetted onto the water surface. After 24 hours of quiescent contact, the solvent layer was removed, the aqueous phase was analyzed and the root surface area index was determined. The mass of each compound that partitioned into the hexane and ethyl acetate decreased with increasing root mass and increasing root surface area index. Partition coefficients calculated with respect to the solvent phase decreased with increasing root mass. These data show that roots can effectively compete against solvents for the partitioning of chlorophenol. The presence of roots simultaneously promoted retention of the chlorphenols in the aqueous phase and provided a sorption site. Differences in results between the two solvents diminished with increasing root mass.

INTRODUCTION

The use of plants to enhance in situ remediation of soils contaminated by organic pollutants is a newly emerging technology. Regardless of the amount of research conducted in the interactions between plants and organic pesticides, many questions remain concerning the use of plants to remediate soil containing organic contaminants (Cunningham, et al., 1996). One question concerns the ability of roots to compete with soil organic matter for organic contaminants through physico-chemical interactions.

Earlier work has demonstrated that the presence of rye grass roots in the aqueous phase reduces the movement of a chlorophenol from the aqueous
solution into an adjacent hexane layer. Part of this decreased partitioning may be attributed to adsorption and absorption of the compound by the roots. The previous work was conducted using single contaminants in the experimental system. Most sites contain multiple compounds. By decreasing the number of sites available for the other compounds, each contaminant may interfere with the adsorption of the others. Thus, one goal of this experiment was to determine if the presence of multiple chlorophenols affected the distribution of each compound between the roots and an adjacent organic phase.

One question that arose from the earlier work is whether the nature of the second organic phase (hexane) affected the distribution of the chlorinated phenols between the roots and the adjacent liquid. Soil organic matter is not as nonpolar as hexane. The ability of roots to reduce sorption in a soil system may depend on the relative polarities of the roots and the soil organic matter. Thus, another goal of this study was to determine if increasing the polarity of the second organic phase would affect the reduction in liquid-liquid partitioning caused by the roots. To accomplish this goal, hexane and the more polar ethyl acetate were used as the second organic phase in the system.

METHODS AND MATERIALS

The experimental solutes were 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP) and pentachlorophenol (PCP). These structurally similar compounds encompass a range of polarity (log $K_{ow}$ from 2.75 to 5.01) and dissociation constant (pK$_a$ from 4.74 to 7.85) (Westall, et al., 1985; Table 5-1). The most hydrophobic compound should yield the greatest extent of movement out of the aqueous phase. However, the most hydrophobic experimental compound (PCP) is characterized by the lowest pK$_a$. From a hydrophobicity perspective, the observed degree of partitioning should be PCP > TCP > DCP. Since the dissociation of the chlorophenols under mildly acidic conditions is greatest for the most hydrophobic compound, the relative extent of partitioning for the test compounds might not follow the pattern predicted by hydrophobicity alone.
Table 5-1. Properties of the Experimental Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Weight (mg/L)</th>
<th>Solubility (mg/L)</th>
<th>pKa</th>
<th>log Kow</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCP</td>
<td>164</td>
<td>7.85</td>
<td>2.75</td>
<td></td>
</tr>
<tr>
<td>TCP</td>
<td>197</td>
<td>800</td>
<td>6.01</td>
<td>3.38</td>
</tr>
<tr>
<td>PCP</td>
<td>266.5</td>
<td>14</td>
<td>4.74</td>
<td>5.01</td>
</tr>
</tbody>
</table>

The second organic phase in the experimental system was either hexane or ethyl acetate. Ethyl acetate is substantially more polar than hexane. Ethyl acetate dissolves to some extent in water and, as a bulk liquid, can form a separate phase that is less dense than water.

Rye grass seeds were germinated in semisolid bactoagar. After two to three weeks of growth, the grasses were removed from the agar and the roots were severed from the shoots. Different amounts of roots, ranging from less than 100 mg to 1400 mg, were weighed and placed into 40 mL, screwtop vials containing 20 mL of a chlorinated phenol/dilute nutrient solution adjusted to either pH 4.9 or pH 6.3. The three compounds were combined in the same solution. For the ethyl acetate treatments, the initial concentration of each chlorophenol ranged from 0.0138 mM to 0.0150 mM. For the hexane treatments, the pH 4.9 solution contained 0.0226 mM DCP, 0.0283 mM TCP and 0.0335 mM PCP, while the pH 6.3 solution contained 0.0179 mM DCP, 0.0161 mM TCP, and 0.0208 mM PCP. Ten mL of organic liquid were pipetted immediately onto the water surface. The phases were not mixed together. Each treatment had duplicate, rootless controls.

After 24 hours of contact time, the solvent layer was removed by pipette and analyzed for chlorophenol content on a Hewlett Packard 5890A Gas Chromatograph fitted with an electron capture detector, an autosampler and a Supelco DB5 column. The aqueous chlorophenol concentration was determined as described below. The root surface area index was measured using the method described in the following section.

Five mL of each aqueous sample was placed into an 11 mL screwtop vial and acidified to below pH 2 with 4 drops of 1 N sulfuric acid. Three mL of hexane was pipetted onto the aqueous phase. The two phases were allowed to contact for 24 hours, after which the hexane was removed by Pasteur pipette.
Next, one mL of the hexane extract was placed in an autosampler vial and the remaining hexane volume was measured. The extracts were analyzed on a Hewlett Packard 5890A Gas Chromatograph fitted with an electron capture detector, an autosampler and a Supelco DB5 column. The sample standard deviation for extraction and analysis was 12.1% for DCP (n = 30), 5.37% for TCP (n = 25), and 6.89% for PCP (n = 25).

**Root Surface Area Index**

Measurement of the adsorption of rhodamine red by roots was used to determine relative surface areas of the samples (Sorenson and Wakeman, 1996). The roots were placed into 125 mL Erlenmeyer flasks containing 30 mL of a rhodamine red solution with a concentration of 2 µM and at a pH of 4.9. The flasks were agitated for 18 hours on a shaker table to achieve equilibrium prior to centrifugation at 10,000 rpm for 10 minutes. The absorbance of the centrate was measured on a spectrophotometer at a wavelength of 550 nm. The adsorbed mass, expressed as umoles, is referred to throughout the paper as the root surface area index and reflects the relative adsorptive capacity of the different samples.

**RESULTS**

Regardless of whether the organic liquid were hexane or ethyl acetate, the grass roots substantially reduced the movement of the three chlorinated phenols into the organic liquid. For all three chlorophenols, the amount in the organic liquid was inversely related to root surface area index and root mass (Figures 5-1 to 5-4). In addition, a number of samples for each treatment contained aqueous chlorophenol concentrations greater than those observed in the corresponding rootless controls (Figures 5-1 to 5-4). Mass balances demonstrated that the three compounds sorbed to the roots.
Figure 5-1. Distribution of the three chlorophenols among the three phases. Solvent phase was hexane and aqueous solution was at pH 4.9.
Figure 5-2. Distribution of the three chlorophenols among the three phases. Solvent phase was hexane and aqueous solution was at pH 6.3.
Figure 5-3. Distribution of the three chlorophenols among the three phases. Solvent was ethyl acetate and aqueous solution was at pH 4.9.
Figure 5-4. Distribution of the three chlorophenols among the three phases. Solvent was ethyl acetate and aqueous solution was at pH 6.3.
Hexane

The addition of roots decreased the mass fraction that partitioned into the hexane in an approximately linear manner with respect to root surface area index (Figures 5-1a and 5-2a). In the samples with the highest amounts of roots, the fraction in hexane was substantially reduced. For example, at pH 4.9, the addition of 0.826 grams of roots with a surface area index of 0.0613 µmoles adsorbed rhodamine red caused the TCP fraction in hexane to reduce from 88.8% of the total mass observed in the rootless controls to 25.5% of the total mass. At both pH 4.9 and pH 6.3, the three compounds yielded similar fractions in the hexane (Figures 5-1a and 5-2a). For each chlorophenol, the pH 6.3 samples tended to yield higher hexane fractions than the pH 4.9 samples (data not shown).

In a previous study, it was observed that the addition of small to medium amounts of roots increased the aqueous concentration of chlorinated phenols. In the current study, an increase in the aqueous concentration upon the addition of roots was also observed (Figures 5-1b and 5-2b). As the amount of roots in solution increased, the concentration increased to a maximum and then decreased (this same trend was observed in the previous study cited). In the current study, DCP exhibited the greatest increase in the aqueous fraction upon the addition of roots, while the PCP aqueous fraction exhibited only a slight increase in the aqueous fraction at the lowest root surface area index and tended to decrease with increasing root surface area (Figures 5-1b and 5-2b).

At pH 4.9, the compound with the greatest fraction in the aqueous phase was DCP (Figure 5-1b). The TCP and PCP aqueous fractions in the same samples were relatively close in value. For TCP and PCP, the aqueous fractions were typically greater at pH 6.3 than pH 4.9, as one would expect from the higher degree of ionization associated with higher pH's. DCP, for which the dissociated fraction does not substantially increase from pH 4.9 to pH 6.3, yielded a smaller fraction in the aqueous phase at pH 6.3 than at pH 4.9.

Substantial amounts of each chlorophenol associated with the grass roots. DCP at pH 4.9, likely due to the increased aqueous concentration in the presence of roots, yielded the lowest fraction associated with the roots, with a maximum of
23.3% of the total mass (Figure 5-1c). In the same treatment, up to 57.7% of the TCP and 63.4% of the PCP sorbed to the roots (Figure 5-1c). At pH 6.3, up to 50% of the DCP and TCP and 64.8% of the PCP associated with the roots (Figure 5-2c). Except for DCP at pH 4.9, the amount of chlorophenol associated with the roots was directly related to both root mass and root surface area index (Table 5-2). At pH 4.9, PCP had the greatest root-associated fraction while DCP had the lowest (Figure 5-1c). At pH 6.3, TCP and DCP had similar root-associated fractions while PCP had the highest (Figure 5-2c). Both TCP and PCP were characterized by greater root-associated fractions at pH 4.9 than at pH 6.3.

Table 5-2. Correlation coefficients for the root-associated mass with respect to root mass or root surface area index.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root Mass</th>
<th>Root Surface Area Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane, pH 4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCP</td>
<td>0.575</td>
<td>0.324</td>
</tr>
<tr>
<td>TCP</td>
<td>0.984</td>
<td>0.879</td>
</tr>
<tr>
<td>PCP</td>
<td>0.946</td>
<td>0.923</td>
</tr>
<tr>
<td>Hexane, pH 6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCP</td>
<td>0.908</td>
<td>0.891</td>
</tr>
<tr>
<td>TCP</td>
<td>0.950</td>
<td>0.937</td>
</tr>
<tr>
<td>PCP</td>
<td>0.951</td>
<td>0.939</td>
</tr>
<tr>
<td>Ethyl Acetate, pH 4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCP</td>
<td>0.842</td>
<td>0.861</td>
</tr>
<tr>
<td>TCP</td>
<td>0.958</td>
<td>0.941</td>
</tr>
<tr>
<td>PCP</td>
<td>0.828</td>
<td>0.848</td>
</tr>
<tr>
<td>Ethyl Acetate, pH 6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCP</td>
<td>0.868</td>
<td>0.857</td>
</tr>
<tr>
<td>TCP</td>
<td>0.933</td>
<td>0.930</td>
</tr>
<tr>
<td>PCP</td>
<td>0.583</td>
<td>0.584</td>
</tr>
</tbody>
</table>

To characterize the affinity of each chlorophenol for the hexane phase, partition coefficients were calculated by dividing the hexane layer concentration by the aqueous phase concentration. Except for PCP at pH 6.3, the hexane $K_p$'s were inversely correlated with root surface area index and root mass (Table 5-3). At pH 4.9, in the rootless controls, TCP tended to have the greatest $K_p$ while DCP typically yielded the lowest. With increasing root content, the $K_p$'s became
similar. The addition of roots decreased the DCP and TCP hexane $K_p$’s by approximately 80%. The lowest PCP hexane $K_p$ was approximately 60% of the control value.

Table 5-3. Correlation coefficients for solvent partition coefficients with respect to root mass or root surface area index.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root Mass</th>
<th>Root Surface Area Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane, pH 4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCP</td>
<td>-0.811</td>
<td>-0.863</td>
</tr>
<tr>
<td>TCP</td>
<td>-0.855</td>
<td>-0.883</td>
</tr>
<tr>
<td>PCP</td>
<td>-0.606</td>
<td>-0.663</td>
</tr>
<tr>
<td>Hexane, pH 6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCP</td>
<td>-0.639</td>
<td>-0.622</td>
</tr>
<tr>
<td>TCP</td>
<td>-0.707</td>
<td>-0.747</td>
</tr>
<tr>
<td>PCP</td>
<td>0.228</td>
<td>0.163</td>
</tr>
<tr>
<td>Ethyl Acetate, pH 4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCP</td>
<td>-0.866</td>
<td>-0.861</td>
</tr>
<tr>
<td>TCP</td>
<td>-0.819</td>
<td>-0.811</td>
</tr>
<tr>
<td>PCP</td>
<td>-0.613</td>
<td>-0.663</td>
</tr>
<tr>
<td>Ethyl Acetate, pH 6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCP</td>
<td>-0.537</td>
<td>-0.562</td>
</tr>
<tr>
<td>TCP</td>
<td>0.0486</td>
<td>-0.0335</td>
</tr>
<tr>
<td>PCP</td>
<td>0.0300</td>
<td>0.0435</td>
</tr>
</tbody>
</table>

**Ethyl Acetate**

In the controls, the fraction of each chlorophenol in the ethyl acetate was considerably less than the corresponding fractions observed in the hexane treatments. For example, at pH 6.3, the rootless ethyl acetate fraction for DCP was 49.0% while the equivalent hexane fraction was 80.3%. Regardless, the addition of grass roots reduced the movement of each chlorophenol into the ethyl acetate by as much as 65% of that observed in the rootless controls. At both pH’s, TCP yielded the greatest fraction in the ethyl acetate phase, while the DCP and PCP values were similar (Figures 5-3a and 5-4a). For DCP and TCP, below a root surface area index of 0.04 umoles adsorbed rhodamine red, the pH 4.9 treatment yielded a greater ethyl acetate fraction (Figure 5-5). Above the root surface area index, the ethyl acetate fractions were similar for both pH’s.
Figure 5-5. Comparison of the partitioning of DCP and TCP into ethyl acetate in the presence of roots at pH 4.9 and pH 6.3.

The aqueous concentration followed the same trend as described for the hexane treatments (Figures 5-3b and 5-4b). In general, DCP had the greatest aqueous fraction of the three compounds at both pH’s (Figures 5-3b and 5-4b). For both DCP and TCP, the aqueous fraction was greater at pH 4.9 than pH 6.3 below a root surface area index of 0.04 umoles of adsorbed rhodamine red. Above that value, the aqueous fractions were similar for both pH’s. The PCP aqueous fraction was consistently greater at pH 6.3 than pH 4.9.

At the lower root surface area indices, the aqueous concentration for each chlorophenol tended to increase relative to that of the rootless controls. At some point, a maximum was reached and the addition of more roots coincided with a decrease in the aqueous concentration. As with the hexane treatment, DCP exhibited the greatest increase in the aqueous concentration. However, as a percent of the control concentration, the samples did not yield as great an increase in the ethyl acetate treatments as compared to the hexane treatment. For example, the maximum DCP aqueous concentration at pH 4.9 was only 1.7 times that of the rootless control. For each pH, DCP was characterized by the greatest increase in concentration. The compounds demonstrated a smaller percentage increase in the
aqueous concentration at pH 6.3 than pH 4.9. In addition, the maximum concentration for each compound occurred at a lower root surface area index at pH 6.3 than at pH 4.9. Thus, the treatment with the higher fraction of ionized chlorophenols resulted in a smaller relative increase in the aqueous concentration.

Substantial amounts of each chlorophenol associated with the grass roots. Up to 38% of the DCP, 47% of the TCP and 68% of the PCP sorbed with the roots (Figures 5-3c and 5-4c). The root-associated fraction tended to be positively correlated with root mass and root surface area index (Table 5-2). At pH 4.9, the fraction associated with the roots decreased with decreasing chlorophenol hydrophobicity (Figure 5-3c). At pH 6.3, the three compounds were characterized by similar root-associated fractions (Figure 5-4c). Compared on a root surface area index basis, DCP and TCP had similar root-associated fractions at both pH’s. PCP yielded a greater root-associated fraction at pH 4.9 than at pH 6.3.

Ethyl acetate K_p’s were calculated for each chlorophenol. At pH 4.9, the chlorophenol ethyl acetate K_p’s were inversely correlated with both root surface area index and root mass (Table 5-3). At pH 6.3, the ethyl acetate K_p’s were not correlated with either root surface area or root mass (Table 5-3). In the presence of roots, the ethyl acetate K_p for DCP decreased by as much as 85%, reducing from 3.65 for the rootless controls to 0.539 at pH 4.9 and from 2.47 for the rootless controls to 0.488 at pH 6.3. In general, TCP had the highest K_p of the three compounds. Below a root surface area index of 0.04 umoles of adsorbed rhodamine red, the K_p’s for DCP and TCP were greater at pH 4.9 than at pH 6.3. Above that root surface area value, the K_p’s were similar at both pH’s. A similar pattern held for PCP, except that the root surface area index value was 0.06 umoles of adsorbed rhodamine red.

Comparison of Ethyl Acetate and Hexane Results

In samples containing low to moderate amounts of roots, the solvent fraction of each chlorophenol was greater for hexane than for ethyl acetate (Figures 5-6 and 5-7). As the root content increased, the solvent fractions became
similar for the two organic liquids. The initial difference in the solvent fractions between the two organic liquids was greater at pH 6.3 than at pH 4.9.

Figure 5-6. Comparison of the movement of TCP into each solvent in the presence of roots, pH 4.9.

Figure 5-7. Comparison of the movement of TCP into each solvent in the presence of roots, pH 6.3.

The ethyl acetate treatments were characterized by greater aqueous fractions than the hexane treatments at both pH’s (Figures 5-8 and 5-9).
Regardless of the increase in the aqueous fraction caused by the addition of low to moderate amounts of roots, the ethyl acetate samples demonstrated a greater propensity to maintain the solute in solution.

Figure 5-8. Comparison of the aqueous TCP fraction in the presence of roots adjacent to either hexane or ethyl acetate, pH 4.9.

Figure 5-9. Comparison of the aqueous TCP fraction in the presence of roots adjacent to either hexane or ethyl acetate, pH 6.3.
The hexane treatment samples tended to have a greater root-associated fraction than the ethyl acetate samples (Figure 5-10). This trend was observed for both pH’s. The $K_p$’s for hexane tended to be greater than those for ethyl acetate in samples characterized by a root surface area index less than 0.04 umole rhodamine red adsorbed (Figure 5-11 and 5-12). Above a root surface area index of 0.04, the $K_p$’s for both solvents were similar.

![Figure 5-10](image-url) **Figure 5-10.** Comparison of the TCP root-associated fraction. Roots in an aqueous solution adjacent to hexane or ethyl acetate. Solution pH of 4.9 or 6.3.

![Figure 5-11](image-url) **Figure 5-11.** Comparison of DCP and TCP $K_p$'s for each solvent, pH 4.9.
Comparison of Chlorophenols Singly in Solution and Combined in Solution

The amount of each individual chlorophenol that associated with the roots was reduced by the presence of other chlorophenols (Figure 5-13). Simultaneously, the individual chlorophenol aqueous concentration was lower in the presence of other chlorophenols than when singly in solution (Figure 5-14). As a result, the hexane partition coefficient for each compound was greater in the presence of other chlorophenols than singly in solution. These results suggest that competition for sorption sites decreased somewhat the ability of the compounds to reduce partitioning into the hexane phase. However, as the hexane results clearly demonstrate, even in the presence of multiple chlorophenols, the roots substantially decrease the movement of each compound into a second organic phase.

Figure 5-12. Comparison of TCP Kp's for each solvent, pH 6.3.
Figure 5-13. DCP mass that associated with roots was lower in the presence of TCP and PCP as compared to DCP as the sole chlorophenol in solution, pH 4.9.

Figure 5-14. The DCP aqueous concentration was higher when it was the sole chlorophenol in solution than when combined with TCP and PCP, pH 4.9.
DISCUSSION

Based on the results and the literature, two mechanisms appear to simultaneously operate within the experimental system: aqueous interactions among cosolutes; and sorption reactions.

The movement of nonpolar compounds out of water into an organic phase results from the energy required to maintain the nonpolar compound dissolved in a hydrogen-bonded matrix (Schwarzenbach, et al., 1993). By interacting with other hydrophobic compounds, organic cosolutes may decrease partitioning by reducing the energy required to maintain the nonpolar compound dissolved in water (Schwarzenbach, et al., 1993). Chiou demonstrated that the aqueous solubility of hydrophobic organic compounds was higher in octanol-saturated water than in pure water (as cited in Schwarzenbach, et al., 1993, p. 134). During the contact period between the chlorophenols and the organic phases, the severed roots released dissolved organic material into solution, even turning the water dark brown in samples containing high root masses. The increase in the chlorophenol aqueous concentration upon the addition of roots relative to the rootless controls suggests that the dissolved material released by the roots provided a cosolute effect.

The higher aqueous fraction observed in the ethyl acetate rootless controls as compared to the hexane controls was due to a cosolute effect, similar to the results obtained by Chiou (as cited in Schwarzenbach, et al., 1993, p. 134). Hexane does not dissolve in water, while ethyl acetate has an approximate 15% solubility in water. Thus, the ethyl acetate samples contained substantial amounts of ethyl acetate in the aqueous phase. The addition of soluble root material simply added to the cosolute effect already present in the ethyl acetate treatment. However, the relative increase in the aqueous concentration caused by the roots was greater for hexane than for ethyl acetate because the hexane controls had no cosolute effect. The percentage reduction in the aqueous activity coefficient was greater when roots released dissolved compounds into an aqueous solution free of cosolutes (hexane samples) than into an aqueous solution already affected by cosolutes (ethyl acetate samples).
This difference in the cosolute effect between the two organic liquids also explains the lower $K_p$’s observed for ethyl acetate as compared to hexane. The convergence of the $K_p$’s at the higher root masses suggests that the greatest reduction in the aqueous activity coefficients of the chlorophenols occurs upon the initial addition of cosolutes. As the cosolute concentration increases, the effect on the aqueous activity coefficient and subsequent effect on partitioning decreases. This observation suggests that, in soils, in which the soil water typically has a high solute concentration initially, the addition of plant roots may not further increase this cosolute effect. Thus, the primary influence of roots in a soil may be through the addition of sorption sites that compete with the soil organic matter for the nonpolar contaminants.

Sorption interactions in the soil are well-documented in the literature. Sorption to the soil organic matter creates obstacles to site remediation through diminished bioavailability and slow desorption (Ogram, et al., 1985). These results demonstrate that roots were able to substantially reduce the movement of each chlorophenol into a second organic phase in the presence of other chlorophenols, even though the three chlorophenols were competing for sorption sites. Thus, the roots should demonstrate some ability to reduce sorption of individual compounds to soil organic matter in soils containing multiple contaminants. Sorption, similar to partitioning, is a function of the aqueous activity coefficient of the compound (Schwarzenbach, et al., 1993). A decrease in the aqueous activity coefficient decreases the driving force resulting in sorption. However, even in the ethyl acetate samples, the roots decreased the movement into the ethyl acetate by as much as 65% of that for the rootless controls. This result indicates that, even in soil systems that have a high cosolute concentration in the soil water, the roots should be able to decrease the movement of nonpolar contaminants onto the soil organic matter.

Thus, the addition of roots have the potential to cause an increase in the aqueous concentration through cosolute interactions, while, through sorption, decreasing the movement of nonpolar compounds onto or into alternate organic phases present in the system. The cosolute effect appears to function over a
limited range of conditions (such as no or minimal cosolute concentration initially in the aqueous solution). Even in the hexane treatment, for which the cosolute effect was most pronounced, the sorption mechanism becomes dominant at high root concentrations, as demonstrated by the reduction in the aqueous concentration in samples containing high amounts of roots.

CONCLUSION

Roots can substantially decrease the movement of multiple contaminants into a second organic phase. If plants were present at a new spill site, their roots would reduce the amount of contaminants that adsorb to soil organic matter. Due to the purely physico-chemical nature of the interactions, this beneficial effect should occur even when the plants are dormant. Placement of plants, such as grasses with very fine root systems that would not affect structures, near tanks, pipelines and other sites of potential contamination should minimize the obstacles to remediation of future contamination. If the contaminant is translocated or transformed within the plant, the plant would further promote remediation. Thus, placement of plants at potential contamination sites would essentially be an installation of a before-the-fact, in situ remediation system. By the time any contamination occurred, the plant roots would be well-established, reducing lag times associated with the typical approach of after-the-fact remediation, such as identifying the extent of contamination, and designing and installing a remediation system.

By adsorbing compounds from contaminated groundwater, an extensive system of roots may form a screen to retard offsite contaminant transport. This potential plant root effect could be exploited to reduce subsurface transport from nonpoint source pollution. Regardless of the specific use, the plants should be selected to maximize the volume of soil exposed to the root surfaces. A species with fine, extensive roots should be the most effective at competing with soil organic matter for dissolved contaminants.
REFERENCES


CHAPTER 6: CARBON ADDITION REDUCED LAG TIME FOR 2,4,6-TRICHLOROPHENOL DEGRADATION

ABSTRACT

The effect of grass root exudates and glucose on the lag time associated with 2,4,6-trichlorophenol (TCP) degradation by an unacclimated microbial inoculant and an acclimated microbial inoculant was investigated. The experimental medium was a nutrient solution containing TCP spiked with radiolabeled TCP and amended with either glucose or root exudates collected from fescue grass. Treatments containing TCP as the sole organic carbon source for the microbial inoculants served as biotic controls. The acclimated microbial inoculant was not provided with TCP for four weeks prior to the experiment. The presence of an alternate organic carbon source reduced lag time for both the acclimated microbial inoculant and the inoculant that had not been previously exposed to chlorinated phenols. For the latter, three ratios of alternate organic carbon content to inoculant biomass were tested. The lag time for acclimation depended on this ratio. A lag time in excess of 11 days was observed for the biotic controls. The shortest lag time with the addition of an alternate organic carbon source was 2 days for glucose and 5 days for root exudates. It is proposed that the presence of a readily available, alternate organic carbon source affected lag time through promotion of microbial population growth and provision of a preferred source of carbon and energy.

INTRODUCTION

Although in situ bioremediation is considered to be an effective remediation technique, its application may be characterized by practical difficulties. One difficulty is the length of time required for the microbes to acclimate themselves to metabolism of certain organics. Several studies have demonstrated that the presence of plant roots and/or their products enhance the
biodegradation of organic contaminants (Hsu and Bartha, 1979; Federle and Schwab, 1989; Walton and Anderson, 1990; Ferro, et al., 1994; Boyle and Shann, 1995; and Burken and Schnoor, 1996).

The specific role that the organic carbon released from roots may play is disputed. One possible effect is facilitation of the acclimation of microbes to metabolism of the specific xenobiotic. This possibility is supported by Hsu and Bartha’s research in which bush bean root exudates stimulated mineralization of parathion in a soil void of actual plant roots. Burken and Schnoor (1996) observed that in soils amended with poplar exudates more atrazine was mineralized than in soils amended with acetate, suggesting that the molecular structure of the organic matter added to the soil affected contaminant degradation. On the other hand, Ferro, et al. (1994), considered trivial the proposition that root-derived organic compounds alone could enhance contaminant mineralization.

The purpose of this study was to determine if the presence of an alternate organic carbon source was sufficient to reduce the length of time required for an unacclimated microbial inoculant to begin mineralization of an organic contaminant (lag time). The research examined 2,4,6-trichlorophenol (TCP), a toxic compound found at wood preserving/processing facilities that can be used as a sole carbon source and that can be completely degraded under aerobic conditions (Robinson, 1990), as the organic contaminant. Two alternate organic carbon sources were compared: soluble organic matter collected from the roots of growing fescue grass and glucose.

METHODS AND MATERIALS

All experiments used stock solutions of non-radiolabeled TCP spiked with a small volume of carbon-14 (C\textsuperscript{14}) labeled TCP. The radiolabeled TCP was synthesized in the lab using C\textsuperscript{14} aniline purchased from Sigma (Robinson, 1990). The radioactivity of each sample was analyzed on a Beckman LS-3150T (analytical error of 2.67%).

Fescue grass seeds were germinated in jars containing semisolid bactoagar. After germination, the seedlings were transferred to a dilute nutrient
solution and allowed to grow for three to four weeks. Then, the solution was analyzed for total organic carbon (TOC) content on a Horiba PIR-2000 and stored at 4°C Celsius until use on the following day.

The acclimated inoculant was cultured from soil (Bhandari, 1995) and maintained in solution containing TCP. Preliminary tests confirmed the ability of the microbial stock to live on TCP as the sole carbon source under aerobic conditions. This acclimated culture was not provided with TCP for four weeks prior to the experiment. The unacclimated microbial inoculant was obtained from lab scale reactors fed a mixture of acetate and isopropyl alcohol. This inoculant was removed from the reactors immediately prior to the start of each experiment. The concentration of the biomass in the reactors was quantified as mg/L suspended biomass, and will be referred to as biomass.

**Acclimated Inoculant**

For the acclimated inoculant experiment, four treatments were used: biotic controls with approximately 10 mg TCP/L; and microbes and soluble grass root products with approximately 1, 10 and 40 mg TCP/L. The intent of this experiment was threefold: first, as a preliminary experiment, to determine if the glassware would be appropriate for use in subsequent studies; second, to determine if the TCP concentration affected microbial activity; and third, to ascertain that the root exudates would yield some effect. Each treatment consisted of two uninoculated flasks to record system loss and five inoculated replicates, except for the biotic controls which had three inoculated replicates. Fifty mL of each stock solution were pipetted into 250 mL screwtop, Erlenmeyer flasks and inoculated with acclimated microbes. Duplicate 250 μL were withdrawn on days 0, 3 and 6.

The results from this preliminary experiment indicated that the carbon dioxide traps for the 250 mL flasks were ineffective. For this reason, the subsequent study with the unacclimated inoculant used different glassware and a sacrificial sampling approach (to minimize system losses and allow accurate measurement of CO₂ evolution). In the abiotic controls, the solution retained 92.1
± 5.41% of the initial TCP at day three and 85.5 ± 4.34% of the initial TCP at day six. To calculate the average and standard deviation values, the percent of initial radioactivity retained by each of the eight abiotic controls, two for each treatment, was pooled together. The results of the acclimated inoculant study are provided in terms of solution activity instead of mineralization.

**Unacclimated Inoculant**

For the unacclimated microbial inoculant studies, 25 mL sidearm flasks with center-well CO\(_2\) traps were obtained. The initial TCP concentrations were 5 mg/L. Each 25 mL, sidearm flask was fitted with a center well containing 250 µl of 1 N potassium hydroxide to trap CO\(_2\). The inoculant was obtained from laboratory reactors not exposed to chlorinated phenols. After inoculation, each flask was sealed with parafilm and silicone. The incubation times ranged from two to 18 days (determined as the experiment proceeded by the observed amount of mineralization). At sampling, 5 N sulfuric acid was injected through the sidearm stopper. After 24 hours, the CO\(_2\) traps were removed and analyzed for radioactivity. Aqueous radioactivity was measured before and after filtration through a 0.45µ filter. The system recovery of radioactivity was 85.9 ± 7.49% (n = 100).

For unacclimated inoculant study, the treatments consisted of the addition of either root exudates or glucose to a solution containing radiolabeled TCP and a microbial inoculant not previously exposed to TCP. The following treatments were used: abiotic controls (TCP only, no inoculant); biotic controls (inoculant with only TCP); soluble grass products added at ratios of 1.06, 2.83 and 3.60 mg TOC/mg biomass; and glucose added at ratios of 1.06, 2.83 and 3.60 mg TOC/mg biomass (Table 6-1). Duplicate abiotic controls flasks and three flasks for each treatment were sacrificed at each sampling. The mineralization of TCP was monitored by the entrapment of radiolabeled carbon dioxide (CO\(_2\)).
Table 6-1. Biomass and concentration of the alternate organic carbon source for the unacclimated inoculant studies.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TCP (mg/L)</th>
<th>Root Exudate or Glucose Concentration (Measured as mg/L TOC)</th>
<th>Biomass (mg/L)</th>
<th>TOC/Biomass (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abiotic Control</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Biotic Control</td>
<td>5</td>
<td>0</td>
<td>6.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>6.89</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td>18.4</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>18.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

RESULTS

Acclimated Inoculant

After three days, the biotic controls contained between 75.0% and 83.9% of the initial radioactivity in solution (Figure 6-1). At this time, the flasks which received soluble grass root products along with TCP contained an average of 33.0 ± 1.98 % (n = 5), 35.9 ± 6.47 % (n = 5) and 36.9 ± 2.13 % (n = 5) of the initial activity in solution for the 1.11, 11.4 and 40.8 mg/L TCP grass root product treatments, respectively. The initial TCP concentration exerted little apparent effect on the percent degradation. By day 6, the amount of C¹⁴ remaining in solution was similar for all treatments.
Figure 6-1. TCP was removed more quickly in samples containing root exudates as compared to samples containing TCP as the sole carbon source.

**Unacclimated Inoculant**

In the abiotic controls, little of the radioactivity was absorbed by the CO$_2$ traps. On day 11, 14.5% and 13.5% of the radioactivity initially in solution had been retained by the CO$_2$ traps of the abiotic controls. Otherwise, less than 8.3% of the initial radioactivity was trapped in the abiotic control CO$_2$ traps. For the biotic controls, only one sample exhibited mineralization. This mineralization was observed in one of the day 18 samples. Thus, in the absence of an alternate organic carbon source, the lag time for the unacclimated organisms exceeded 11 days and may have been greater than 18 days.

For the grass exudate treatments, the highest exudate TOC/biomass ratio appeared to yield the shortest lag time, somewhere between five and nine days (Figure 6-2). The 1.06 mg TOC/mg biomass and 2.83 mg TOC/mg biomass samples began TCP mineralization between days seven and eleven. By the end of the experiment, the different treatments exhibited a similar extent of
mineralization, ranging from 43.8% to 56.1% of the initial radioactivity found in the CO₂ traps.

![Graph showing CO₂ production over incubation time]

Figure 6-2. Addition of grass root exudates decreased lag time as compared to biotic controls containing TCP as the sole carbon source.

With the addition of glucose (Figure 6-3), the lowest TOC/biomass ratio resulted in the shortest lag time, two days, of all the experimental treatments. The lag period for the 2.83 mg TOC/biomass treatment was between seven and eleven days. The lag period for the 3.60 mg TOC/mg biomass treatment was between nine and twelve days. For the three glucose treatments, the extent of mineralization was within the range observed for the grass product treatments.
Figure 6-3. Addition of glucose decreased lag time as compared to biotic controls containing TCP as the sole organic carbon source.

**DISCUSSION**

The starved, acclimated microbial inoculant was stimulated by the addition of a second carbon source. Since the biotic controls removed an average of 10% of the solution activity beyond system loss within the first three days, the microbial inoculant had retained its ability to metabolize TCP. However, to metabolize TCP, it was necessary for the microbes to shift from a resting/decay state to an actively growing state. This transition required a combination of time and metabolic energy. Plant roots produce simple organic compounds that can readily enter the metabolic pathways of aerobic microorganisms and stimulate their population growth (Tate III, et al., 1991). Thus, the addition of plant root products shortened the transition time to rapid metabolism of TCP most likely by increasing the number of microbes present.

Comparison of the biotic controls to the glucose and soluble grass root product treatments demonstrates that the addition of a second energy and carbon
source stimulated metabolic acclimation of microbes not previously exposed to TCP. It has been proposed that the molecular structure of plant-derived organic compounds causes the microbial community to shift to favor organisms capable of degrading those types of molecules (Holden and Firestone, 1997). Similarity between the molecular structures of plant compounds and the molecular structures of xenobiotics allows the microbes to use the enzymes synthesized for plant material degradation on the organic contaminants (Boyle and Shann, 1995; Holden and Firestone, 1997). Since glucose, which bears little molecular resemblance to TCP, yielded the shortest lag time, the observed enhanced acclimation cannot be described by a shift in the microbial community to favor phenol degraders. The other explanation is that the microbial population increase supported the adaptation. This “priming effect” has been offered by Boyle and Shann (1995) as a possible means by which root exudates affect degradation of soil contaminants.

With the addition of glucose, lowest TOC/biomass ratio was associated with the most rapid acclimation (Figure 6-3). On the other hand, with the addition of root exudates, the highest TOC/biomass ratio appeared yield the shortest lag time (Figure 6-2). It is possible that the alternate organic carbon sources serves two functions: promotion of microbial population growth; and provision of a preferred source of carbon and energy. This proposition suggests that an optimum or effective TOC/biomass ratio exists for this contaminant in either a soil or wastewater matrix. Since all of the glucose is available for immediate use by the microbial inoculant, less glucose would be required to minimize lag time as compared to the soluble root products that are comprised of a variety of compounds, some of which are not readily available to the microbial population. Only a fraction of the TOC in the grass product treatments would have been immediately available to support initial population growth.

If the amount of readily available organic carbon is very small relative to the inoculant biomass (very low ratio of TOC/biomass), it is unlikely that the alternate organic carbon source would exert an appreciable effect on population growth. As the ratio increases, population would be stimulated, reducing the lag
time. Since the readily oxidizable carbon (e.g., glucose, low molecular weight organic acids) would likely be preferred over TCP as a substrate, the microbes will consume most of the alternate carbon source prior to synthesizing and/or utilizing the enzymes required for TCP metabolism. As the effective TOC/biomass ratio continues to increase, the amount of preferred substrate becomes so great that the microbes require more time to consume the alternate organic carbon prior to switching to TCP metabolism. Thus, as TOC/biomass ratio increase from zero, the lag time reduces to a minimum and then increases.

The presence of a readily oxidizable, alternate organic carbon source reduced the lag time necessary for a previously unexposed microbial inoculant to adapt to TCP metabolism. The data demonstrate that, with respect to TCP, the alternate carbon source need not be structurally similar to TCP in order to yield a beneficial effect. These results suggest that, at a site characterized by a low organic carbon content, the addition of a readily oxidizable, alternate organic carbon source may decrease acclimation time. This beneficial effect is supported by the findings of Nair and Schnoor (1994), who observed greater atrazine mineralization in soils with higher organic matter contents. With respect to aerobic degradation of contaminants that can be used as a sole carbon source, such as TCP, this research suggests that the specific source of the alternate organic carbon, whether from plants or as glucose, is not as important as its readily metabolizable nature.

In addition, these data suggest that a larger microbial population will beneficially affect the onset of microbial degradation of organic contaminants in soils even if the microbial population has not been previously exposed to the compound(s). Further research in this area could lead to more cost-effective approaches to in situ bioremediation. Such research may also lead to a re-evaluation of laboratory experiments in which a carrier organic, such as methanol, was used to add the contaminant of concern to the soil. The addition of a readily degradable organic carrier may affect the degradation results of the xenobiotic under investigation.
It is recommended that further research be conducted to explore the effects of root exudates and other organic carbon sources on the degradation of organic contaminants. A greater range of TOC/biomass ratios should be tested and the effect of different alternate organic carbon sources should be examined.

REFERENCES


VITA

Cynthia Crane was born in Concord, Mass., in 1967. She received a B.A. in Economics from the University of New Hampshire. In 1992, she earned a M.S. in Environmental Engineering from Virginia Tech. She completed her Ph.D. in Civil Engineering at Virginia Tech in 1999. In 1998, she started a Juris Doctor program at The George Washington University Law School. She intends to pursue a career in environmental law.