Arch. Environ. Contam. Toxicol. 50, 191–195 (2006)
DOI: 10.1007/s00244-004-0119-8

ARCHIVES OF
Environmental
Contamination
and Toxicology

Remediation of Soil Contaminated with 2,4-Dichlorophenol by Treatment of Minced Shepherd's Purse Roots

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Received: 31 May 2004 /Accepted: 13 September 2004

Abstract. This study investigated the applicability of minced shepherd's purse root containing high peroxidase activity as a catalytic agent instead of purified and immobilized peroxidase for the remediation of soil contaminated with phenolic pollutants, using 2,4-dichlorophenol (2,4-DCP) as a model pollutant. The removal of 2,4-DCP in the soil was extremely fast when treated with peroxide and minced shepherd's purse root, and maximal removal was achieved within 10 min. Increasing the reaction temperature did not significantly influence removal of 2,4-DCP incubated with shepherd's purse. The removal of 2,4-DCP was dependent on the amount of shepherd's purse and the soil moisture content. Increasing the amount of shepherd's purse and moisture mixed with soil caused an increased removal of 2,4-DCP. Calcium peroxide was more effective than hydrogen peroxide, and maximal removal was achieved in 20 mM of both peroxides. The efficiency of 2,4-DCP removal decreased with increasing 2,4-DCP concentration but was greater than 60% at 500 to 1000 mg kg^{-1}. Our results provide strong evidence that shepherd's purse can be used to remediate soil contaminated with phenolic pollutants.

Because soil is the ultimate receptor of contaminants and the diffusion and dilution of contaminants is very difficult, the detoxification or remediation naturally occurring is very slow (Decaprio 1997). Therefore, contaminants often persist, producing potentially harmful effects on the environment.

Most phenols, which are introduced from paper mills, dye works, and minefields, are carcinogenic and are considered to be endocrine disrupters (Kovacs *et al.* 1993; O'Connor *et al.* 1992).

In the past decade, it has been suggested that phenols might be detoxified through oxidative coupling, which is related to humification in the soil environment, in the presence of oxidoreductive enzymes such as peroxidase, laccase, and tyrosinase (Bollag 1992; Dec and Bollag 1990, 1994; Pal *et al*. 1994; Stevenson 1994). However, purified peroxidase may be limited for the remediation of soil, because purified peroxidase is unstable under severe field conditions (Cao *et al*. 1996). To solve this problem, the enzymes have been immobilized on clays or glass beads. The immobilized enzymes are more stable to temperature change and proteolysis than free enzymes and also effectively detoxified phenols and aromatic amines in a broader pH range (Bollag 1992; Cao *et al*. 1996; Gianfreda and Bollag 1994; Leonowicz *et al*. 1988; Sarkar *et al*. 1989). However, there is a disadvantage for the immobilization of enzymes such as peroxidase on matrices. The immobilization of enzymes is expensive, and the procedure is relatively complicated. Additionally, a considerable amount of enzymes can be deactivated during the immobilization process.

On the other hand, several researchers (Alder and Splar 1994; Dec and Bollag 1994, 1995; Kim *et al*. 1995; Lee *et al*. 1999, 2000; Roper *et al*. 1996) have suggested the use of plant materials to detoxify organic contaminants in soil and aqueous environments. Dec and Bollag (1994) suggested that minced horseradish could be used to remove xenobiotics in wastewater. They found that similar results occurred in the transformation of xenobiotics when compared with purified horseradish peroxidase. Recently, Lee *et al*. (1999) found that peroxidase contained in shepherd's purse root was about fourfold more effective than horseradish root for the removal of organic pollutants. They reported that peroxidase contained in shepherd's purse root successfully removed various phenols and aromatic amines in the presence of hydrogen peroxide in an aqueous solution. Furthermore, minced shepherd's purse can be reused several times and used throughout a broader pH range to detoxify phenols and aromatic amines due to protection of peroxidase from deactivation by the plant tissue (Lee *et al*. 2000).

The objective of this study was to evaluate the applicability of minced shepherd's purse root to remediate soil contaminated with organic pollutants, such as phenols, aromatic amines, and pesticides and their metabolites, using 2,4-dichlorophenol (2,4-DCP) as model compound. Various reaction conditions affecting removal rate in soil environments were evaluated.

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Table 1. Physicochemical properties of the soil used

	Particle size dist. (%)						
Texture	Clay	Silt	Sand	pH (1:5)	Organic matter(%)	CEC (cmol kg^{-1})	Moisture (%)
Silty clay	35.0	44.1	20.9	6.7	1.3	9.1	2.9

CEC = Cation Exchange Capacity.

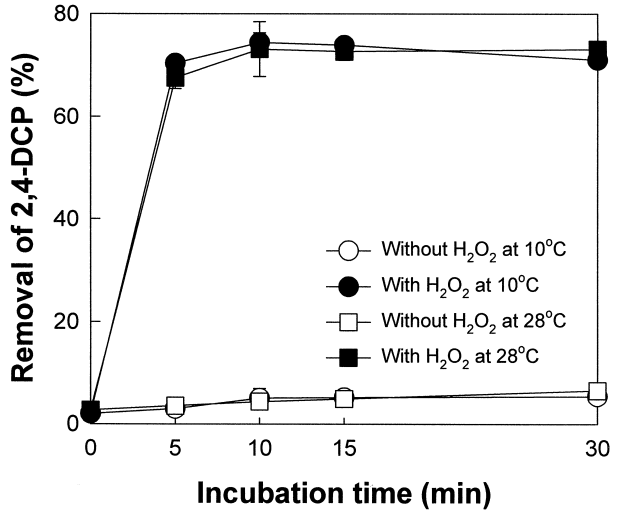

Fig. 1. Time required to removal of 2,4-DCP in 5 g of soil on two different temperatures in the presence of shepherd's purse.

Materials and Methods

Shepherd's purse was purchased from a farm market in Korea. Roots were cut and collected, washed with water, minced, and stored under −20°C before use. Substrate, 2,4-DCP, was obtained from Sigma Co. (St. Louis, MO, USA). The soil used was collected from an upland field in Chilgok, Daegu, Korea (an experimental field of Agriculture College, Kyungpook National University). The collected soil was air dried, passed through a 2-mm sieve, and stored at 4°C. The physicochemical properties of the soil used are presented in Table 1.

Five grams of soil placed in a 50-mL brown bottle was treated with 500 mg kg^{-1} of 2,4-DCP. This soil was mixed with 1.0 g of minced shepherd's purse root, watered as much as 100% of WHC (water-holding capacity), and incubated at room temperature for 3 hours in the presence or absence of 20 mM of peroxide. Soil mixed with boiled shepherd's purse served as control. All experiments were conducted in triplicate.

To evaluate the effect of incubation time on the removal of 2,4-DCP incubated with shepherd's purse, the soil was incubated for different times (0, 5, 10, 15, and 30 min). The soil samples were acidified with 500 μL of 2 M HCl to stop reaction after incubation. To investigate the effect of incubation temperature, soil samples were incubated at 10 and 28°C. The shepherd's purse was mixed with soil in amounts ranging from 0 to 2 g to evaluate the effect of amount of shepherd's purse used on the removal of 2,4-DCP in soil and incubated in described conditions above. Two types of peroxide (hydrogen peroxide and calcium peroxide) were used to compare the role of electron acceptor in the removal reaction of 2,4-DCP. To evaluate the effect of an added amount of peroxide on the removal of 2,4-DCP incubated with shepherd's purse, each peroxide was used in amounts ranging from 0 to 30 mM per 5 g of soil. Soil samples prepared with the above-described experimental conditions were moistened from 25 to 200% of their maximum WHC and incubated for 3 h to determine the effect of soil moisture on the removal of 2,4-DCP incubated with shepherd's purse in soil. To investigate the effect of initial substrate concentration on its own removal, 2,4-DCP was added to 5 g of soil to obtain concentration of 50, 100, 300, 500, 1000, and 2000 mg kg^{-1}.

After incubation, soil samples were extracted with 20 mL of methanol for 1 h in a shaker and centrifuged for 10 min at 12,000 rev min^{-1}. The supernatant was filtered through a 0.45-μm nylon (Millipore Corp., Milford, MA, USA) filter and analyzed with an HPLC system using a Shimadzu-10A HPLC system (Shimadzu Co., Kyoto, Japan) equipped with an S 5200 sample injector (Sykam Gmbh, Eresing, Germany) and a UV absorbance detector operated at 280 nm using a 300 X 3.9 mm μ-Bondapak^{TM} C_{18} analytical column of 5-μm particle size with a 2-cm guard column (Waters Co., Milford, MA, USA). The mobile phase for analysis of 2,4-DCP was composed of methanol and water containing 2% acetic acid and 0.018 M ammonium acetate at a ratio of 40/60 (methanol/water, v/v) at a flow rate of 1.0 mL per minute.

Results and Discussion

The effect of incubation time and temperature on the removal of 2,4-DCP from soil incubated with shepherd's purse and hydrogen peroxide is presented in Figure 1. Maximal removal of 2,4-DCP was achieved within 10 min. However, no significant increase occurred thereafter. In the absence of peroxide as a control, 20% of 2,4-DCP was removed due to physical sorption into the tissue of plant materials. A similar result was observed when 2,4-DCP was incubated with boiled shepherd's purse in the presence or absence of peroxide (data not shown).

The result showed that the reaction initiated with shepherd's purse was terminated within 10 min. Lee *et al.* (2000) observed that shepherd's purse could be reused many times for the treatment of wastewater contaminated with phenolic compounds. Therefore, it is likely that the hydrogen peroxide used to initiate the reaction was consumed within 10 min of incubation and the reaction will be reactivated if another peroxide dose is added.

In previous studies, it was determined that the removal of substrates incubated with free oxidoreductive enzyme was dependent on the reaction temperature (Park *et al.* 1999). However, when shepherd's purse was used, no significant change in the removal of 2,4-DCP occurred due to an increase of the reaction temperature from 10°C to 28°C. This result suggests that shepherd's purse can be used to remediate organic pollutant regardless of changes in soil temperature with season.

The removal of 2,4-DCP was significantly increased from 0 to 75% of initial concentration when the amount of shepherd's purse added to the soil was increased from 0 to 2 g per 5 g of

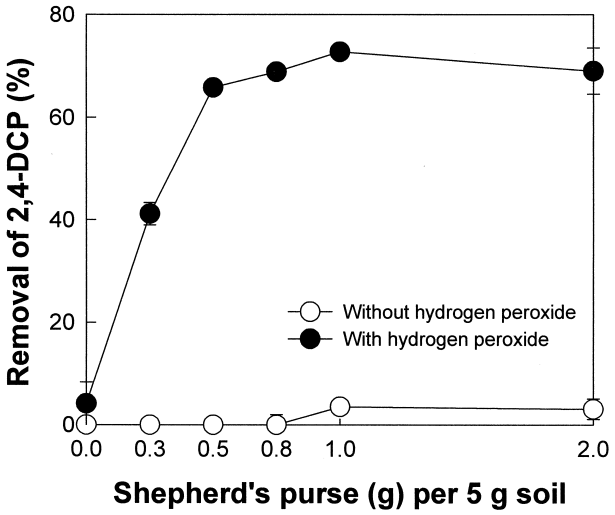

Fig. 2. Effect of shepherd's purse amount added on the removal of 2,4-DCP during a 3-hour incubation in 5 g of soil.

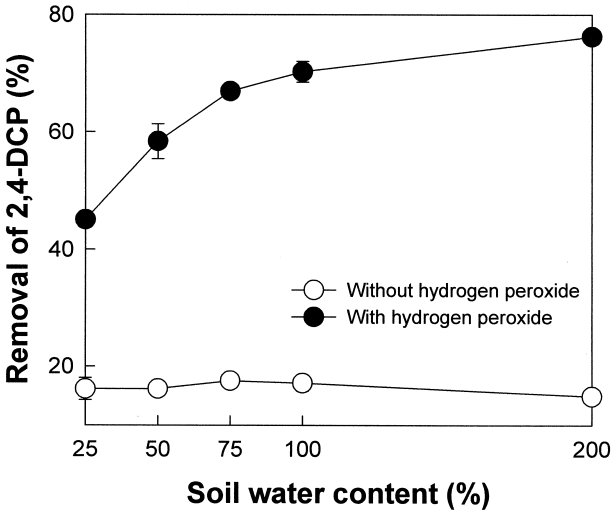

Fig. 3. Effect of soil moisture content on the removal of 2,4-DCP during a 3-hour incubation in 5 g of soil.

soil (Figure 2). Maximum removal of 2,4-DCP was achieved by adding 1 g of shepherd's purse to 5 g of soil. When the amount of shepherd's purse was increased to 0.5 g, the removal of 2,4-DCP was rapidly increased. However, further increasing the amount of shepherd's purse caused a negligible increase (less than 5%) in the removal of 2,4-DCP. This is probably due to insufficient peroxide available for further reaction. Therefore, it may be expected that an increase in the removal rate of 2,4-DCP will occur with another dosage of peroxide.

To investigate the effect of soil moisture content on the removal of 2,4-DCP incubated with shepherd's purse, the content of soil moisture was increased from 25% to 200% of the WHC (Figure 3). Removal of 2,4-DCP increased from 45% of the initial concentration at 25% WHC to 78% at 200% WHC. This observation indicates that the moisture content of soil is one of the most important factors for the removal of 2,4-

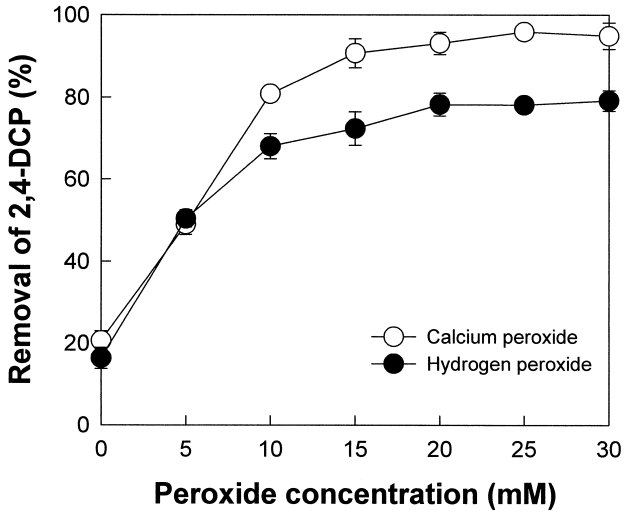

Fig. 4. Effect of type and amount of peroxide added on the removal of 2,4-DCP during a 3-hour incubation in 5 g of soil.

DCP incubated with shepherd's purse and peroxide. The contact between shepherd's purse, peroxide, and 2,4-DCP will increase with increasing soil water content. To achieve effective remediation, a sufficient water supply is necessary.

The removal of 2,4-DCP from soil was affected by the type and amount of peroxide used. In the presence of shepherd's purse, increasing hydrogen peroxide concentration from 5 to 30 mM increased 2,4-DCP removal from 18% to 78%, while calcium peroxide increased removal from 20% to 95% of initial concentration (Figure 4). The maximal removal was achieved in 20 mM of both peroxides (hydrogen peroxide and calcium peroxide) mixed with 1 g of shepherd's purse per 5 g of soil. However, no additional significant increase was observed when more peroxide was added up to 30 mM. On the other hand, no significant removal of 2,4-DCP was observed in control incubated with boiled shepherd's purse or without peroxide (result described above). This result suggests that the removal of 2,4-DCP is mediated by the peroxidase present in the shepherd's purse, and that peroxidases require the peroxide supplied by electron receptors to initiate reaction.

In a previous study, Bollag and Dec (1990) suggested that an excess of peroxide can decrease removal of a substrate incubated with peroxidase by partial deactivation of enzyme. However, in this study, no deactivation of the enzyme was observed within 30 mM of peroxide.

The type of peroxide had an effect on the removal of 2,4-DCP incubated with shepherd's purse. In the presence of shepherd's purse, calcium peroxide was more effective than hydrogen peroxide in the removal of 2,4-DCP by 16.7%. This trend could be explained on the basis of the physicochemical properties of CaO_2 as observed in the previous study. In case of CaO_2, more effective utilization of the peroxide by the shepherd's purse is facilitated by slow dissolution of CaO_2 to H_2O_2 in the aqueous phase of the soil (Flanders *et al.* 1999). Additionally, Ca^{2+} regulates the activation of a secretary process that releases peroxidase (Casterline *et al.* 1985). Therefore, the addition of CaO_2 may increase the content of peroxidase available, thus releasing more peroxidase from the

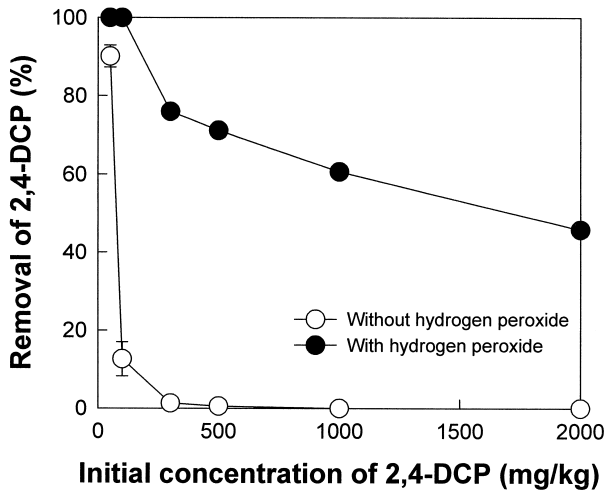

Fig. 5. Effect of initial substrate concentration on the removal of 2,4-DCP during a 3-hour incubation in 5 g of soil.

shepherd's purse in the aqueous phase. Another possible explanation is the pH change by CaO_2 in soil from 6.7 to 8.5. At a higher pH, the structural stability of 2,4-DCP, which has a pKa of 7.85, would be decreased and promote interaction between 2,4-DCP, and superoxide ions and hydroxylic radicals, of which shepherd's purse induced the formation.

Figure 5 illustrates the effect of initial concentration of 2,4-DCP on its removal in the presence of shepherd's purse. The removal of 2,4-DCP was decreased when the initial concentration of 2,4-DCP was increased. This phenomenon could be explained by the results of Wu *et al.* (1998). They suggested that phenolic oligomers formed through oxidative coupling of substrate could deactivate peroxidase. However, despite a decreased removal of substrate at a high initial concentration, using shepherd's purse can be considered an alternative method for remediation of soil contaminated with organic pollutants.

Previously, we tried to purify the enzymes present in shepherd's purse, and purified enzyme was identified to peroxidase according to the formation of colored polymers of 2,6-dimethoxyphenol, which was used as a substrate in the determination of specific peroxidase activity in the presence of hydrogen peroxide. We found that the peroxidase activity of shepherd's purse was fourfold greater than that of horseradish (Lee *et al.* 1999).

In previous studies, a number of researchers suggested that toxic organic compounds were detoxified through oxidative coupling mediated with oxidoreductive enzymes found in various bacteria and fungi (Shuttleworth *et al.* 1986; Xu 1996; Yaver *et al.* 1996) and higher plants (Alder and Splar 1994; Dec and Bollag 1994, 1995; Kim *et al.* 1995; Lee *et al.* 1999, 2000; Roper *et al.* 1996). The oxidative coupling by oxidoreductive enzymes participates in the humification of various phenolic substances that are produced through the decomposition of lignin in a soil environment (Stevenson 1994). In the same way, oxidoreductive enzymes can detoxify toxic xenobiotics, such as phenolic or anilinic toxic compounds, through polymerization, co-polymerization with other substrates, or binding to humic substances (Bollag *et al.* 1980; Dec and Bollag 1990; Hatcher *et al.* 1993; Pal *et al.* 1994; Shannon and Bartha 1988; Simmons *et al.* 1989; Sjoblad and Bollag 1981; Stevenson 1994).

In this study, we observed that shepherd's purse can effectively remove 2,4-DCP up to 95% of the initial concentration in soil. Because the removal of 2,4-DCP was less 20% of initial concentration in control samples treated only with shepherd's purse or peroxide, it clearly indicated that the removal of 2,4-DCP was due to enzyme activity present in shepherd's purse.

Although the detoxification pathway of 2,4-DCP by shepherd's purse was not investigated in this study, it may be assumed that 2,4-DCP was polymerized through coupling to itself on the basis of results obtained from previous investigation using free enzymes (Sjoblad and Bollag 1981; Bollag *et al.* 1980; Simmons *et al.* 1989; Tatsumi *et al.* 1994).

On the other hand, it appears that immobilization of phenolic compounds in soil is due to covalent binding to humic substances through oxidative coupling mediated by oxidoreductive enzymes (Bollag *et al.* 1980; Hatcher *et al.* 1993). Therefore, some 2,4-DCP incubated with shepherd's purse could also be covalently bound to humic substances through oxidative coupling.

Based on the results of previous studies (Park *et al.* 2000; Sakar *et al.* 1988; Shannon and Bartha 1988), 2,4-DCP incubated with shepherd's purse was removed not only by polymerization but also by binding to humic acid in a soil environment. No doubt, binding to soil humic substance through direct binding and cross-coupling with humic constituent monomers and polymerization through coupling to itself can occur simultaneously in soil.

Toxic compounds, either bound to humic substance or polymerized, exhibit reduced toxicity, bioavailability, and mobility in soil (Bollag 1997). Therefore, the transformation of toxic compounds through oxidative coupling mediated with shepherd's purse may result in detoxification in soil.

In conclusion, shepherd's purse containing peroxidase can effectively mediate the removal of 2,4-DCP in soil regardless of soil pH and temperature. Therefore, it can be recommended that application of shepherd's purse is an appropriate method for the remediation of toxic organic compounds such as phenolic compounds, aromatic amines, and some pesticides and their metabolic intermediates in soil.

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