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Impacts of heavy metals on 1,2-dichloroethane biodegradation in co-contaminated soil

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Abstract

1,2-Dichloroethane (DCA), a potential mutagen and carcinogen, is commonly introduced into the environment through its industrial and agricultural use. In this study, the impact of lead and mercury on DCA degradation in soil was investigated, owing to the complex co-contamination problem frequently encountered in most sites. 1,2-Dichloroethane was degraded readily in both contaminated loam and clay soils with the degradation rate constants ranging between 0.370–0.536 week^{-1} and 0.309–0.417 week^{-1}, respectively. The presence of heavy metals have a negative impact on DCA degradation in both soil types, resulting in up to 24.11% reduction in DCA degradation within one week. Both biostimulation and treatment additives increased DCA degradation, with the best degradation observed upon addition of glucose and a combination of diphosphate salt and sodium chloride, leading to about 17.91% and 43.50% increase in DCA degradation, respectively. The results have promising potential for effective remediation of soils co-contaminated with chlorinated organics and heavy metals. However, the best bioremediation strategy will depend on the soil types, microbial population present in the soil matrices, nutrients availability and metal forms.

Key words: biodegradation; biostimulation; co-contamination; heavy metals; treatment additives

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Introduction

The frequent production and increased use of enormous quantities of chlorinated organic compounds has led to their widespread distribution in the environment (Fetzner, 1998). They are introduced into the environment through their use as pesticides (Boyle *et al*., 1999), from effluents of pulp and paper industries, as well as from bleaching plants and chlorination procedures used in the treatment of potable water, wastewater and cooling water (Yu and Welander, 1995). 1,2-Dichloroethane (DCA), in particular, is produced annually in excess of 5.443×10^9 kg per year, a volume larger than that of any other industrial halogenated chemical (Janssen *et al*., 1989). It is used as a chemical intermediate in the synthesis of a number of other chlorinated hydrocarbons, especially vinyl chloride which is used to make a variety of plastic and vinyl products including polyvinyl chloride (PVC) pipes, furniture upholstery, wall coverings, house wares, and automobile parts (IARC, 1999; IPCS, 1995). In the past, it has been used as grain and soil fumigant, metal degreasers, varnish removers, solvent for cleaning textiles and in organic syntheses (IARC, 1999).

The widespread use of DCA in a variety of products such as trichloroethylene and tetrachloroethane, and manufacturing processes has made it to be frequently encountered in most sites contaminated with organic chemicals (Defra and Environment Agency, 2002; Hage and Hartmans, 1999) and has been indicated at least 570 of the 1585 National Priorities List sites identified by the Environment Protection Agency (Adriano *et al*., 1998). The major concern over soil contamination with DCA stems largely from health risks, both of direct contact and from secondary contamination of water supplies. Owing to their toxicity, persistence and potential for bioaccumulation (Squillace *et al*., 1999). There is therefore a growing interest in technologies for the removal of DCA because of their toxic effects on humans and environment (Baptista *et al*., 2006; van den Wijngaard *et al*., 1993).

1,2-Dichloroethane is susceptible to both abiotic and biological transformation and its aerobic biodegradation has been intensively studied. Microorganisms capable of transforming DCA into innocuous products have also been identified and studied (Jeffers *et al*., 1989; Janssen *et al*., 1985; Stucki and Leisinger, 1983). However, soils co-contaminated with organics and heavy metals are considered difficult to remediate because of the mixed nature of contaminants, which must be treated differently (Sandrin and Maier, 2003). The presence of heavy-metal ions at high concentrations can result in the formation of unspecific complex compounds in the cell, which leads to

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toxic effects thus, inhibiting the activity of microorganisms involved in degradation of organics (Roane *et al*., 2001). Metal toxicity is most commonly attributed to the tight binding of metal ions to sulfhydryl (–SH) groups of enzymes essential for general microbial metabolism or those directly involved in biodegradation, thus rendering the enzyme nonfunctional (Sandrin and Maier, 2003). The presence of metals can therefore inhibit a broad range of microbial processes including dehalogenation, and reductive processes involved in DCA degradation (Said and Lewis, 1991).

In spite of the complex problems presented by co-contamination in most contaminated sites, the effects of metal toxicity on organic pollutant biodegradation in co-contaminated soil environments have not been adequately defined due to the fact that metals can be present in a range of different physical and chemical forms such as colloidal solutions, soluble complexes or organic solutes (Sandrin and Maier, 2003). Previously, we described the aerobic biodegradation of DCA by some bacterial isolated indigenous to contaminated sites in South Africa (Olaniran *et al*., 2007). Therefore, the focus of this study was to assess the impacts of heavy metals on the aerobic biodegradation of DCA in co-contaminated soil undergoing bioremediation by the indigenous soil organisms. The potential of certain nutrients and treatment additives to enhance the degradation process was also investigated. This is expected to assist in determining the best bioremediation option needed for such co-contaminated sites, especially in areas widely polluted with this group of toxic compounds and heavy metals without appropriate remediation efforts and regulatory acts to monitor their discharge into the environment.

1 Materials and methods

1.1 Sample collection and handling

The soil samples were collected from specific sites in the Westville area of Durban, KwaZulu-Natal, to obtain a representative sample of the indigenous microbial community living attached to the sand grains. The soil types, clay and loam soil, were collected from separate locations and sieved using a 1.7-mm sieve thereafter, stored at 4°C prior to use in the microcosm setup.

1.2 Microcosms setup

Soil microcosms were constructed in a laminar flow cabinet using synthetic groundwater as described by Klier *et al*. (1999). The synthetic groundwater contained (mmol/L) $MgCl_2$ 1.5, KCl 0.12, NH_4NO_3 0.03, $CaCl_2$ 1.0, $Ca(OH)_2$ 1.5, and $NaHCO_3$ 8.5 in deionized water at pH 7.8. The reaction mixtures were prepared by combining 100 g of soil and 75 mL of the synthetic water in a sterile 250 mL serum bottle (Wheaton, USA). Aerobic conditions were maintained by purging the reaction mixture with pure oxygen gas during the preparation, using a 1.2-μm filter and a syringe. Various concentrations, 420, 840, and 1680 mg/kg, of heavy metals, i.e., lead and mercury were then added separately to the different microcosms. For the biostimulation experiment, 1.5 g of glucose, 1.5 g of Kompel fertilizer (Chemicult Products, Pty Ltd., South Africa), an agricultural fertilizer with N:P:K of 3:1:6 and 1.5 g of yeast extract were added separately to each reaction mixture. To determine the effect of treatment additives on DCA degradation, 5 g of calcium carbonate and 1.12 g of disodium phosphate combined with 0.046 g of sodium chloride were added to separate reaction mixture. The microcosms were sealed with sterile, Teflon-lined butyl-rubber stopper and aluminium crimp seal immediately after spiking with DCA. Sufficient reaction mixtures were prepared for each set allowing sampling of the microcosms in triplicates. In order to determine the initial concentration of DCA, the bottles were shaken for 2 h on a rotary shaker at 150 r/min, 25°C to allow for the equilibration of oxygen and DCA between the gas and aqueous phases. Thereafter, the bottles were incubated at 25°C without shaking. Biological inhibited controls were prepared for each set of reaction mixtures using autoclaved soil and included in the study to measure abiotic losses of DCA. The microcosms were sampled weekly over a 3-week period to measure DCA degradation.

1.3 Analytical procedures

The biodegradation of DCA was monitored using a gas chromatograph (Varian model 3700, USA), equipped with a flame ionization detector and a packed column. Ultra high purity nitrogen was used as carrier gas with a flow rate of 10 mL/min. Samples were analyzed with the injector and detector at 200°C and the column at 100°C by injecting 500 μL headspace samples directly into the gas chromatograph, using a gas-tight syringe (Hamilton). Concentrations of DCA were determined by substituting the GC peak areas obtained at the different sampling times into the linear regression equation obtained from a standard curve derived from known quantities of DCA in serum bottles with the same gas and liquid volumes as the experimental bottles. The biodegradation rate constants in each microcosm were estimated according to LaGrega *et al*. (1994).

1.4 Soil analysis

The soil samples were analysed for pH, calcium, magnesium, sodium, iron, nitrate, nitrite, phosphate, sulphate, soluble organic carbon and total Kjeldahl nitrogen using standard methods (Black *et al*., 1965). The moisture content was determined by drying a known quantity of the soil samples at 70°C for 72 h, until a constant weight was obtained. The difference between the initial and final dry weight of the soil gave the moisture content of the soil.

2 Results

2.1 Soil characterization

The chemical properties of both loam and clay soil samples used in the microcosm experiments are listed in Table 1. The pH of the two soil samples was very

Table 1 Physicochemical properties of the soil samples

Determinant	Loam soil	Clay soil
Calcium (μg/g)	1844 ± 46.862	33429 ± 324.709
Magnessium (μg/g)	1450 ± 35	1968 ± 17.088
Sodium (μg/g)	326 ± 15.524	260 ± 27.622
Potassium (μg/g)	1426 ± 45.530	2734 ± 46.293
Iron (μg/g)	3140 ± 35	20700 ± 606.403
Nitrate (soluble) (μg/g)	22.1 ± 1.253	< 0.5
Nitrite (soluble) (μg/g)	< 0.5	< 0.5
Phosphate (μg/g)	146400 ± 556.776	628000 ± 1571.623
Sulphate (soluble) (μg/g)	15.8 ± 2.381	1081 ± 18.520
Soluble organic carbon (μg/g)	264 ± 15.395	21 ± 1.808
Total Kjeldahl nitrogen (μg/g)	1910 ± 40.927	88.4 ± 2.869
pH	5.44 ± 0.695	5.15 ± 0.517
Moisture content (%)	12.64 ± 0.686	14.57 ± 1.492

Values are mean of results from triplicates analysis ± standard deviation

close and ranged from 5.15 to 5.44, while the moisture content of the clay soil was found to be about 15% higher than that of the loam soil. Calcium concentration was found to be about eighteen-fold, potassium about two-fold, iron about seven-fold, phosphate about four-fold, sulphate about sixty eight-fold, and magnesium 35.72% higher in clay soil than in loam soil. However, total Kjeldahl nitrogen concentration was found to be about twenty two-fold, soluble organic carbon about thirteen-fold, nitrate about forty four-fold and sodium 25.38% higher in loam soil than in clay soil. Soluble nitrite was estimated to be approximately < 0.5 μg/g in both soil types (Table 1).

2.2 Impact of different heavy metal concentrations on DCA biodegradation in soil

The degradation rate constants of 1,2-DCA under the different treatment conditions are shown in Table 2. In all cases, except for the soil co-contaminated with 1,2-DCA and 420 mg/kg Pb, higher degradation rate constant was observed in loam soil compared to the clay soil. The degradation of DCA in both contaminated soil samples in the presence and absence of heavy metals is presented in Fig. 1. 1,2-Dichloroethane was observed to degrade readily in both contaminated soil types with higher degradation generally observed in loam soil than in clay soil. The degradation rate constants ranged variously between 0.370–0.536 week^{-1} and 0.309–0.417 week^{-1}, in loam and clay soil, respectively (Table 2) with up to 53.635% degradation (above that of the autoclaved control) observed in loam soil after 2 weeks in the absence of heavy metals. The value is about 10% more than the degradation observed in clay soil at the same period. The presence of heavy metals was observed to have a negative impact on the biodegradation of DCA in both soil samples. The effect was more pronounced in loam soil than in clay soil, resulting in a drastic 21.485%, 23.305% and 24.105% reduction in DCA degradation within one week, in the presence of 840 mg/kg Pb, 1680 mg/kg Hg and the mixture of lead and mercury, respectively. These values are generally about two-fold more than the percentage reduction in DCA degradation observed in clay soil at the same time for the same concentrations of the heavy metals.

An increased reduction in DCA degradation was observed with increasing concentration of the heavy metals. For example, 22.74% reduction in DCA degradation was observed in the presence of 840 mg/kg Pb in loam soil after two weeks while only 13.350% reduction occurred at 420 mg/kg Pb concentration. Similarly, in clay soil, 420 mg/kg Pb concentration resulted in 2.670% decrease in DCA degradation after two weeks while 10.747% reduction was observed at 840 mg/kg Pb. A similar inhibitory effect on DCA degradation was observed at 420 mg/kg Pb and 840 mg/kg Hg, whereas, there was no major difference in the DCA degradation inhibitory effects observed for the mixture of lead and mercury, compared to the single metals in both contaminated soil types.

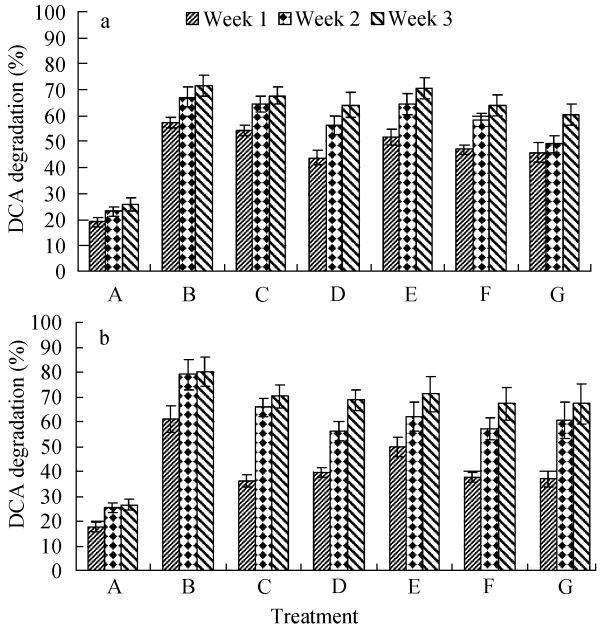

Fig. 1 Biodegradation profile of DCA in clay soil (a) and loam soil (b) co-contaminated with varying concentrations of lead and mercury. A = autoclaved soil control; B = unautoclaved soil + DCA; C = soil + DCA + 420 mg/kg Pb; D = soil + DCA + 840 mg/kg Pb; E = soil + DCA + 840 mg/kg Hg; F = soil + DCA + 1680 mg/kg Hg; G = soil + DCA + 840 mg/kg Pb + 1680 mg/kg Hg). Bars indicate the average of triplicate samples while the error bars show the standard deviation.

2.3 Effect of biostimulation and treatment additives on DCA degradation in cocontaminated soil

Of the three nutrients used in the biostimulation experiments, glucose was found to result in the highest overall degradation of DCA in both soil types, resulting in 80.995% (Fig. 2a) and 82.392% (Fig. 2b) degradation after three weeks in clay and loam soil, respectively. These values correspond to 20.530% and 15.250% increased DCA degradation above those observed in the untreated co-contaminated soil microcosms. Addition of yeast extract and fertilizer resulted in 28.360% and 15.990% increase in DCA degradation, respectively within one week in loam soil (Fig. 2b), while only 5.950% and 6.201% increased DCA degradation was observed in clay soil and only after three weeks (Fig. 2a). In the microcosms setup to determine the effect of certain treatment additives in enhancing DCA degradation in co-contaminated soil, addition of Na_2HPO_4 and NaCl demonstrated a greater effectiveness in improving degradation. In loam soil, 43.497% increased

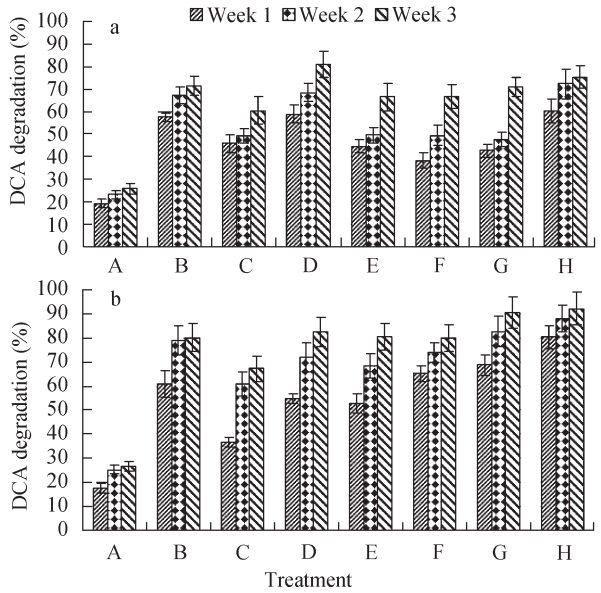

Fig. 2 Effects of biostimulation and treatment additives on the biodegradation of DCA in clay soil (a) and loam soil (b). A = unautoclaved soil control; B = soil + DCA; C = soil + DCA + Pb + Hg; D = soil + DCA + Pb + Hg + glucose; E = soil + DCA + Pb + Hg + fertilizer; F = soil + DCA + Pb + Hg + yeast extract; G = soil + DCA + Pb + Hg + $CaCO_3$; H = soil + DCA + Pb + Hg + Na_2HPO_4 + NaCl. Bars indicate the average of triplicate samples while the error bars show the standard deviation.

degradation was observed upon the addition of Na_2HPO_4 and NaCl in one week (Fig. 2b). After three weeks, DCA degradation in the co-contaminated soil was found to be increased by 24.950% (Fig. 2b). Furthermore, an increase of 31.851% and 23.331% was observed with the addition of $CaCO_3$ during the same degradation period. However, only 14.798% and 10.504% increase in DCA degradation was observed in clay soil after three weeks (Fig. 2a).

Table 2 Biodegradation rate constants ($week^{-1}$) of DCA in soil microcosms under various treatments

Treatment	Clay soil	Loam soil
A	0.0989 ± 0.0170	0.103 ± 0.0131
B	0.417 ± 0.0413	0.536 ± 0.0534
C	0.377 ± 0.0435	0.370 ± 0.0656
D	0.342 ± 0.0520	0.386 ± 0.0156
E	0.407 ± 0.0466	0.413 ± 0.0275
F	0.341 ± 0.0327	0.370 ± 0.0346
G	0.309 ± 0.0269	0.371 ± 0.0415
H	0.553 ± 0.0620	0.579 ± 0.0291
I	0.364 ± 0.0547	0.545 ± 0.0550
J	0.366 ± 0.0475	0.535 ± 0.0625
K	0.412 ± 0.0692	0.784 ± 0.101
L	0.466 ± 0.0463	0.846 ± 0.0681

Values are mean of results from triplicate analysis ± standard deviation. A–H are the same as that in Fig. 1, I = soil + DCA + 840 mg/kg Pb + 1680 mg/kg Hg + fertilizer; J = soil + DCA + 840 mg/kg Pb + 1680 mg/kg Hg + yeast extract; K = soil + DCA + 840 mg/kg Pb + 1680 mg/kg Hg + $CaCO_3$; L = soil + DCA + 840 mg/kg Pb + 1680 mg/kg Hg + Na_2HPO_4 + NaCl.

3 Discussion

Several microorganisms have been characterized for their ability to metabolize DCA under various conditions (De Wildeman *et al*., 2003; He *et al*., 2003; Maymo-Gatell *et al*., 1999; Janssen *et al*., 1985; Stucki and Leisinger, 1983). However, rapid industrial and domestic activities have caused a concomitant increase in the quantities of metals that are being introduced into the environment (Stephen *et al*., 1998), thus, resulting in co-contamination of soil with organic and heavy metal pollutants. This continues to present a more serious and complex problem, as the two contaminants must be treated differently (Sandrin and Maier, 2003; Roane *et al*., 2001). Approximately 40% of the hazardous waste sites currently on the national priority list of the U.S. Environmental Protection Agency are co-contaminated (Sandrin and Maier, 2003).

To assess the impact of heavy metal co-contaminants on DCA degradation, we monitored the aerobic biodegradation of DCA in the presence of varying concentrations of lead and mercury in clay and loam soil microcosm settings. Furthermore, the effects of biostimulation and treatment additives for enhanced DCA degradation in co-contaminated soil were evaluated to determine the possible bioremediation option for removal of DCA in the co-contaminated sites. The results obtained from this study indicate that DCA was readily degraded by indigenous microbial populations in soil microcosms not contaminated with heavy metals and loam soil, exhibiting more degradation than clay soil. However, the presence of heavy metals (lead and mercury) was found to have adverse effects on the biodegradation of DCA by the indigenous soil microorganisms. It has been well documented that the presence of metals can inhibit a broad range of microbial processes, including methane metabolism, growth, nitrogen and sulfur conversions, dehalogenation, and reductive processes in general (Baath, 1989). Metals exert their toxic effects on microorganisms through different mechanisms, including substitution of toxic metal cations for physiologically essential cations within an enzyme, rendering the enzyme nonfunctional (Nies, 1999); and imposition of oxidative stress on microorganisms (Kachur *et al*., 1998). A significant decrease in phenanthrene biodegradation has been previously observed in the presence of cadmium, resulting in the increase of lag growth phase of the organisms involved by 5 d (Maslin and Maier, 2000). Furthermore, heavy metal ions have been reported to be able to form unspecific complex compounds in microbial cells, thus inhibiting microbial activities as a result of toxic effects (Roane *et al*., 2001).

In this study, lead was found to have a greater inhibitory effect on the soil microorganisms involved in DCA degradation than mercury at the concentrations tested, despite the report that mercury is the most toxic of the heavy metals owing to the strong affinity of Hg^{2+} to thiol groups (Nies, 1999). Lead in the ionic form (Pb^{2+}) has been shown to inhibit aerobic biodegradation of crude oil by *Pseudomonas* sp. and *Micrococcus* sp. at 2.80 mg/L and 1.41 mg/L, respectively (Benka-Coker and Ekundayo, 1998). Similarly, anaerobic degradation of hexachlorobenzene has been shown to be inbited by Pb^{2+} at a concentration as low as 0.001 mg/g in microcosms containing contaminated sediments (Jackson and Pardue, 1998). A possible reason for the lower toxicity of Hg on indigenous bacteria, may be

due to the presence of Hg resistance determinants (*mer*) in some of these soil organisms since these genes have been reported to be widespread in most bacteria (Nies, 1999). However, this is the subject of on-going studies in our laboratory.

The physical and chemical properties of soil have been shown to have a reflective influence on aeration, nutrient availability, water retention, and consequently on microbial activity (Olaniran *et al*., 2006). Some of the most important properties are particle size, aeration status, moisture content, pH, organic fraction and the cationic exchange capacity. Clay has been shown to reduce metal bioavailability and toxicity to bacteria, fungi and actinomycetes in solution studies (Babich and Stotzky, 1978). Hence, it is not surprising that the toxicity of the heavy metals used in this study was masked in clay soil, thus resulting in a reduced impact on DCA degradation compared to what was observed in loam soil. The very high concentration of phosphate in the clay soil than in loam soil could also contribute to the less impact of the heavy metals on DCA degradation since phosphates has been shown to reduce solution phase metal concentrations by acting as a metal precipitating agent (Poulson *et al*.,1997; Hughes and Poole, 1991). Also, the observed lesser effect of the combination of metals at high concentrations in clay soil compared to the individual metals. This has been attributed to the fact that these metals are able to form complexes resulting in less bioavailable metal ions (Benka-Coker and Ekundayo, 1998).

Addition of nutrients such as glucose and nitrogen fertilizers has been observed to enhance degradation of contaminants (Mohn and Tiedje, 1992). As a general rule, a ratio of carbon to nitrogen to phosphorus of 100:10:1 (Norris, 1994) has been extensively used in the biodegradation processes (Button *et al*., 1992). In this study, addition of nutrients resulted in enhanced DCA degradation in the co-contaminated soil samples to varying degrees. The high DCA degradation observed upon addition of glucose in the biostimulation experiments is very promising for effective bioremediation of co-contaminated soil since glucose is a simple sugar which is highly soluble in water and therefore easily metabolized by microorganisms (Gao and Skeen, 1998). Minerals or supplementary carbon substrates have been previously demonstrated to increase degradation processes (Loh and Wang, 1998; Fava *et al*., 1995) and according to Sandrin and Maier (2003), yeast extract may serve as a dual function, i.e., a nutrient as well as a metal binding component. In both loam and clay soil, disodium phosphate together with sodium chloride proved to be the better treatment additive for increased DCA degradation. This could be attributed to the possible formation of chloropyromorphite and a reduction in dissolved metal concentrations, thus reducing metal toxicity towards microorganisms required for the degradation (Ruby *et al*., 1994). Treatment additives including phosphate and $CaCO_3$ have been reported to reduce metal bioavailability and mobility (Hettiarachchi *et al*., 2000; Jonioh *et al*., 1999).

4 Conclusions

This study has successfully demonstrated the potential of indigenous microbial populations in co-contaminated loam and clay soils to degrade DCA, under aerobic conditions. Also, the presence of heavy metals at high concentrations has been shown to adversely affect microbial activities in soils, thus resulting in a decrease in DCA degradation. Characterization of the indigenous microbial community to identify the predominant phylotypes involved in DCA degradation, using both culture-dependent and culture-independent methods is the subject of on-going research in our laboratory. Both biostimulation and treatment additives proved to be effective strategies in increasing DCA degradation in the co-contaminated soils, however, the best bioremediation strategy will depend on the soil types, microbial population present in the soil matrices, nutrients available for microbial activities as well as the metal forms. The enhanced DCA degradation upon the addition of nutrients and treatment additives has great and promising potential for effective remediation of soils co-contaminated with chlorinated organics and heavy metals. This is important as most sites encountered are commonly contaminated with a mixture of pollutants.

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