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A comparison of the ability of forest and agricultural soils to mineralize chlorinated aromatic compounds

Roberta R. Fulthorpe* & Linda N. Schofield
*Division of Physical Sciences, University of Toronto at Scarborough, 1265 Military Trail, Scarborough, Ontario, Canada, M1C 1A4 (*author for correspondence; e-mail: fulthorpe@scar.utoronto.ca)*

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Abstract

Soils were sampled from two agricultural fields, two relatively pristine forests, and one suburban forest in Ontario, Canada. The ability of these soils to mineralize 2,4-dichlorophenoxyacetate, 3-chlorobenzoate, 4-chlorophenol, 2,4-dichlorophenol, pentachlorophenol, and atrazine was determined using ^{14}C-labeled substrates. Direct pre-exposure was necessary before atrazine mineralization could be detected; however, it was not necessary for degradation of any of the other chemicals. 2,4-dichlorophenoxyacetate and pentachlorophenol mineralization was much higher in the agricultural soils relative to the pristine forest soils, but 3-chlorobenzoate and 2,4-dichlorophenol mineralization rates showed the opposite trend. Mineralization of 4-chlorophenol was about equivalent in all soils. Suburban forests soils were indistinguishable from agricultural soils with respect to their degradation of 2,4-dichlorophenoxyacetate and chlorobenzoate. Additionally, they were better able than any of the soils to withstand the toxic effects of pentachlorophenol. Pentachlorophenol mineralization was highly variable in the pristine forest soils, ranging from about 6 to 50%. Abiotic factors such as pH, soil type, and organic and moisture content did not account for these significant site differences. The selective forces responsible for these differences, and the possible differences in microbial populations are discussed.

Abbreviations: 3CBA – 3-chlorobenzoate; 2,4-D – 2,4-dichlorophenoxyacetate; PCP – pentachlorophenol; 4CP – 4 chlorophenol; DCP – 2,4-dichlorophenol.

Introduction

The ability of microorganisms to break down xenobiotics such as chlorinated aromatic compounds has long been assumed to be a recently evolved trait, selected for by the presence of pesticides and their residues in the environment. However, it is now clear that the ability to mineralize chlorinated aromatics such as 3-chlorobenzoate (3CBA) and 2,4-dichlorophenoxyacetate (2,4-D) is present in pristine soils that have not been directly exposed to xenobiotics (Fulthorpe et al. 1996). In addition, a high diversity of 3CBA mineralizers have been isolated from such soils (Fulthorpe et al. 1998). These findings are not surprising in the light of the discovery of high levels of natural chloroaromatic compounds produced by a variety of wood-rot fungi (de Jong et al. 1994). However they do appear to conflict with the findings of others that 3CBA degraders in particular are rare (Brurisbach and Reineke 1993, Hernandez et al. 1995; Hickey and Focht 1990, Hickey et al. 1993). We were interested in finding the reason for these conflicting results and hypothesized that they might be due to the use of different methodologies or the study of soils harboring dissimilar microbial communities. Accordingly, the purpose of this study was to apply the same methods to study the mineralization abilities of soils from different ecosystems.

It is reasonable to assume that organic substrates that leach into soils will have a major impact on the species composition and capabilities of the bacterial community. Typically microbiologists have sampled

areas contaminated by anthropogenic chemicals in order to isolate and study the catabolism of key contaminants. Only more recently have we begun to realize that the organic inputs that are derived from the vegetation growing in the soils can have a measurable impact on the metabolic capabilities of the microbial community. Much of this work has been fueled by an interest in phytoremediation and a realization that rhizosphere bacteria specific to certain plants may be effective degraders of specific organic contaminants (Anderson and Coats 1995; Boyle and Shann 1995; Entry and Ebbingham 1995; Sandman and Loos 1984; Siciliano and Germida 1997). Other work has demonstrated that certain vegetative types can select for specific bacterial strains (Fulthorpe et al. 1998).

In this work we compared two agricultural soils with a known history of application of chloroaromatic pesticides with the soils of Great Lakes – St. Lawrence forests from three locations. Two of these forest locations were quite distant from urban areas and uncontaminated by anthropogenic chemicals, while the other was located within a suburban ravine within a metropolitan area. We looked at the ability of these soils to mineralize a suite of 5 chlorinated phenolics and atrazine using radiolabeled substrates, and we found the agricultural soils to have a more highly specific substrate range than the forests soils.

Methods and materials

Sampling sites

The soil samples were collected from three forest sites and two agricultural stations, all in Ontario, Canada. The forests were all typical Great Lakes – St. Lawrence forests, dominated primarily by white pine, hemlock, yellow birch, sugar maple, red maple, balsam poplar, and balsam fir. Two of the forests sites had no direct exposure to any anthropogenic chemicals – Graythorpe, located near Bancroft, 45.1 N 77.7 W; and Springwater Pond Conservation Area located near Aylmer, 42.8N 81.0 W. The third forest site also had no known direct chemical exposures, but it was located close to suburban gardens and parks, where pesticide spraying is common, and was close to roads and footpaths. This site was Highland Creek Ravine on the University of Toronto at Scarborough campus, 43.8 N 79.2 W. The agricultural soils were taken from two research stations: Elora, 43.7 N 80.4 W; and Arkell, 43.5 N 80.4 W. These fields had been planted with corn, wheat, beans, barley, soy, and hay and had a documented history of pesticide exposure, including atrazine, 2,4-D amine and esters, bromoxynil, and monochlorophenoxyacetate. The sites were mapped onto a grid, and random numbers were used to determine sampling locations within each site. Samples were taken using a flame-sterilized metal sampling device and kept in ziplock bags at 4 °C in the dark until use. Samples were composite samples of material from 5 to 20 cm below the surface of the mineral soil, and twelve samples were taken from each site. The sampling sites were determined by using a random number table to choose coordinates from a grid map of each of the sites.

Soil characteristics

The water content of the soil was determined by drying overnight at 105 °C. For pH determinations 1 g of soil was mixed with 5 ml of distilled water and vortexed for 1 minute (pH meter: Orion model 420A). Soil organic carbon contents were determined by ashing dry soils at 550 °C for 1 hour.

Chemicals

Pentachlorophenol (PCP), UL-[^{14}C]PCP (>98%), 4-chlorophenol (4CP), 2,4-dichlorophenol (DCP), UL-[^{14}C]DCP (97%), 3-chlorobenzoate (CBA) 2,4-dichlorophenoxyacetate (2,4D), UL-[^{14}C]2,4D (>98%), UL-[^{14}C]atrazine (95%) and sodium bicarbonate-^{14}C were obtained from Sigma Chemical Co. UL-[^{14}C]CBA (98.1%) and UL-[^{14}C]4CP (98%), from California Bionuclear Corp. (Los Angeles, Calif.) and atrazine (98.7%) from Ciba-Geigy Corp.

Mineralization of labeled compounds

Mineralization studies were carried out by adding 2 g (dry weight equivalent) of soil to sterile 20-ml scintillation vials, and amending them with 50 μg/g of unlabelled substrate and 0.05 μCi of labeled substrate. Atrazine was dissolved in ether, which was left to evaporate after adding it to the soil. Water was then added to these soils to obtain final moisture contents of 25%. A sterile, 1-ml glass vial was filled with 0.5 ml of a 1 M NaOH solution and placed in the scintillation vial as a $^{14}CO_2$ trap. For each chemical tested, experiments were run in triplicate. All vials were kept in containers saturated with water to prevent desiccation. To monitor mineralization activity, NaOH solutions were read and replaced on a weekly basis. The NaOH

was mixed with 10 ml of scintillation cocktail (Beckman, Ready Safe) and 1.5 ml of methanol and vortexed for 10 seconds. The amount of radioactivity was counted for 1 minute in a scintillation counter (Beckman® Instruments Inc., LS6800). To calibrate the amount of $Na^{14}CO_3$ trapped in the solution, aliquots of a labeled $NaHCO_3$ stock solution were put into 1 ml of water in scintillation vials including a vial with 0.5 ml of 1 M NaOH and allowed to dissociate. After one week all the bicarbonate had disassociated to carbon dioxide and was captured in the NaOH solution. Counts of $Na^{14}CO_3$ were regressed against μCi added to water.

To ensure that observed mineralization was due to biotic activity, a control experiment was performed with three replicates for each chemical at 50 μg/g dry soil in which soil was sterilized by autoclaving for 30 minutes. Because relatively high counts were observed from the sterile soil treated with DCP, another experiment at higher concentrations with autoclaved soil was performed. Four replicates were tested, one sample from Highland Creek, Graythorpe, and Springwater and one agricultural sample all at 100, 300, 500 and 750 mg/kg. To determine if mineralization was due to bacterial rather than fungal action, a set of experiments were carried out where fungicides were added to the soil. Nystatin (5 μg/g) was added by dissolving in ether, dispensing over soil surface, and waiting for 1 hour for the ether to evaporate before other amendments were carried out. Cycloheximide (100 μg/g) was added with the water and substrates.

Response of soils to higher concentrations

In order to determine the effects of substrate concentration on mineralization rates, we examined the soil responses to 4 concentrations higher than 50 μg/g. In order to avoid complete inhibition of activity, the minimum inhibitory concentration was estimated by monitoring substrate degradation in liquid media inoculated with soil. Chloroaromatic substrate concentrations were determined using HPLC. These data were used to set up mineralization experiments with 3 replicates at four different concentrations for four compounds.

In order to examine the effect of higher concentrations of the same chemicals, three samples from each site were chosen. The samples that were best able to mineralize the compounds at 50 μg/g were chosen for this. The agricultural samples used for the concentration study were from Elora field 11 for CBA, DCP, and PCP and field 30 for 2,4D, and Arkell field 3 for all compounds.

Table 1. Mean and standard deviation of soil characteristics, from 12 samples per site.

Sampling site	pH	Percent organic	$\%H_2O$
Arkell	7.9 (0.2)	5.5 (0.2)	25 (5)
Elora	8.1 (0.3)	7.7 (0.3)	33 (8)
Springwater	5.9 (0.8)	5.2 (0.2)	25 (8)
Graythorpe	5.9 (0.5)	5.2 (0.3)	30 (24)
Highland Creek	7.9 (0.2)	6.4 (0.4)	30 (17)

Chemical analysis

Liquid chloroaromatic concentrations were determined using a Waters HPLC system that included a NovaPak C18 reverse phase column and a Waters 9600 Photo Diode Array detector. The mobile phase consisted of equal parts of 1% (v/v) phosphoric acid and acetonitrile.

Results

Soil characteristics

The characteristics of the sampled soils are shown in Table 1. The soils had similar organic contents, ranging from 5.5 to 7.7%, and similar moisture contents. The agricultural sites and the Highland Creek forest site were slightly basic, and the two more rural forest sites were slightly acidic.

Mineralization at 50 μg/g dry soil

The correlation between the radioactivity trapped in the NaOH solutions and $^{14}CO_2$ added to the water was significant. The regression line $\mu\text{Ci} = 1.3259 \times 10^{-4} + 3.258 \times 10^{-7} * \text{CPM}$ ($r^2 = 0.983$) was used to convert counts to μCi for determination of mineralization rates. Typical mineralization curves are shown for two of the chemicals in Figure 1. The graphs show some of the main findings of this study. First, there is a large difference in the ability of the soils to mineralize the test compounds. These differences are chemical specific, i.e., one cannot conclude that agricultural soils degrade all chloroaromatic compounds better than do forest soils or vice versa. Second, for all chemicals, average mineralization rates from the Graythorpe

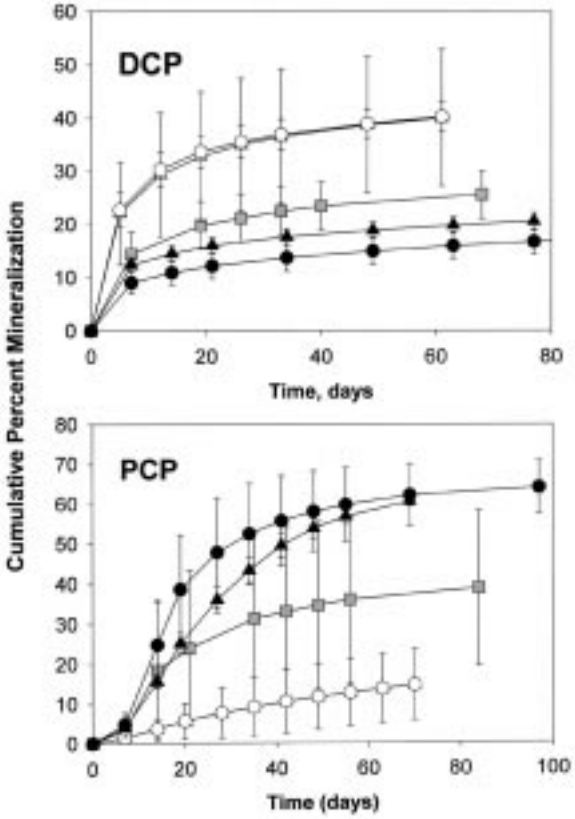

Figure 1. Mineralization of PCP and DCP by forest and agricultural soils. Error bars are standard deviations. Note the quite different behaviour of the soils for each chemical, the intermediate behaviour of Highland Creek (grey squares), and the strong similarity of responses of the Arkell and Elora sites (black triangles and black circles, respectively) and the Graythorpe and Springwater Pond sites (open circles and open triangles, respectively).

and Springwater soils did not significantly differ from each other, nor did average rates from the agricultural sites, Arkell and Elora. Highland Creek forest soils, however, show more variability between samples and behaved like agricultural soils with respect to some chemicals, but like forest soils with respect to others.

The mean mineralization levels achieved after 60 days for all chemicals applied at 50 μg/g dry soil for all sites is given in Figure 2. The forest sites Springwater and Graythorpe mineralized significantly more 3CBA ($p < 0.0001$) and DCP ($p < 0.0001$) than did the agricultural soils, but significantly less PCP ($p < 0.0001$) and atrazine ($p < 0.0001$). The 60-day mineralization rates are comparable for 2,4-D and 4CP. The Highland Creek samples appear to behave like agricultural soils, mineralizing comparable amounts of 3CBA, 2,4-D, DCP and 4CP. However there is no atrazine degradation in Highland Creek forest, and PCP is degradation is midway between that of the agricultural soils and the other more remote forest sites. In all three forest sites PCP mineralization was patchy – ranging from 2.7 to 56% in Highland Creek, from 6.3 to 37% in Graythorpe, and 8.6 to 47.7% in Springwater. It is apparent that bacteria capable of mineralizing PCP can be found in forested systems, but their distribution, or their activity is limited.

Graythorpe and Springwater have lower pHs than Highland Creek forest soils and this might have contributed to differences in mineralization rates (Table 1). Both PCP and 4CP mineralization rates are positively correlated to pH ($r = 0.75$, $r = 0.71$, respectively), while 3CBA and DCP degradation are negatively correlated ($r = -0.83$, $r = -0.81$, respectively). All correlations are significant at $p < 0.01$. There were no significant correlations between mineralization rates of any of the substrates and water content or organic content. After addition of fungicides, mineralization either increased or remained the same suggesting that mineralization is not performed by fungi.

Sensitivity to higher concentrations

Four chemicals were studied at higher concentrations – 2,4D and CBA at 250, 500, 750, and 1000 μg/g, PCP: at 100, 150, 300, 500 μg/g and DCP: at 100, 300, 500, 750 μg/g. The experiments ran up to 71 days, being terminated when no major changes in mineralization were noted (i.e., when curves had leveled off or showed constant slope if linear). Figure 3 illustrates the average amount of chemical mineralized at the end of the experiments. Agricultural soils show very high levels of 2,4-D at all concentrations tested, and Highland Creek mineralization amounts improve at high concentrations. Springwater soils show a drop in mineralization with concentration that is statistically significant, as do Graythorpe soils at the highest concentration tested. The picture is quite the opposite when mineralization of 3CBA is analyzed. The rural forest areas show the highest mineralization amounts at all concentrations, and mineralization inhibition at higher concentrations is not statistically significant. The Highland Creek soils and agricultural soils both show consistently lower CBA mineralization amounts, and statistically significant inhibition above 500 μg/g. The responses to DCP are more similar to CBA – higher mineralization in rural forest soils across the range, but inhibition is evident in all soils except the

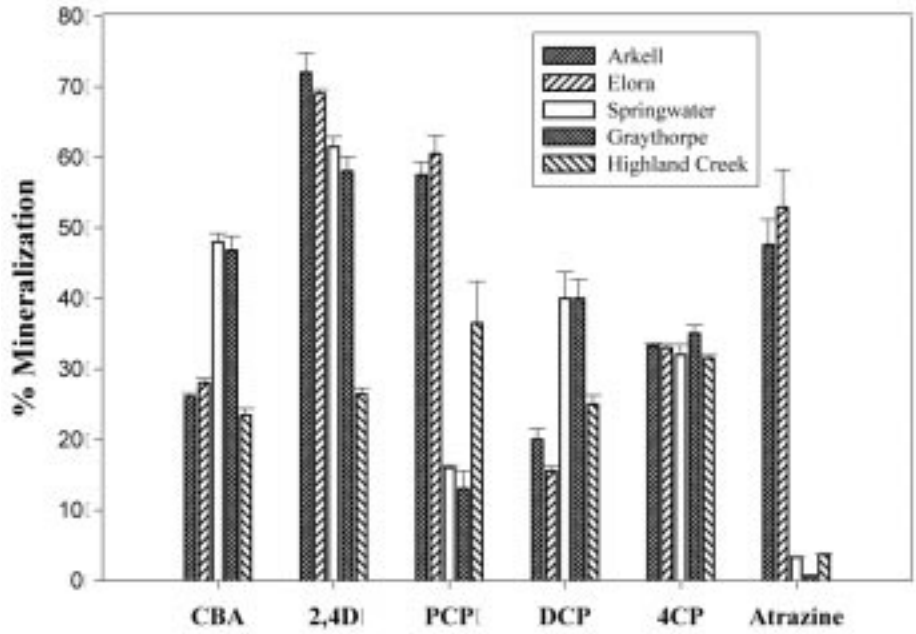

Figure 2. Mineralization of the test chemicals at 50 μg/g dry soil after 60 days. Error bars are standard errors.

Highland Creek soils. PCP mineralization is strongly inhibited by concentrations above 100 to 150 μg/g in all soils tested. Highland Creek soils show much more tolerance to high PCP concentrations than do agricultural soils.

The effect of the different concentrations is more dramatically illustrated by looking at the amount of time each soil took to degrade 25% of the chemical (Figure 4). The higher concentrations typically pushed the mineralization curves to the right, lengthening the lag phase, or changed the curves from logistic to linear, both these effects leading to longer times before 25% mineralization could be achieved. Once again, the clear differences between chemicals and the unique nature of the Highland Creek soils are illustrated. The forest soil degraders are clearly relatively insensitive to high concentrations of both CBA and DCP, while they are quite sensitive to high levels of 2,4-D. The agricultural soils show exactly the opposite sensitivities, with a particularly odd reaction to DCP. The poorest mineralization rates were seen for the lowest concentrations, the highest for intermediate concentrations. Highland Creek behaves like an agricultural soil with respect to 2,4-D and CBA, but like a forest soil with respect to DCP. Of the five soils, those from Highland Creek can degrade the highest concentrations of PCP, followed by the soils of Springwater, while Graythorpe and the agricultural soils exhibit equivalent sensitivities to high concentrations of PCP.

PCP mineralization and pH effects

A more intensive sampling of Graythorpe soils was carried out in order to examine differences in chemical metabolism in soils found directly under different vegetation types and to examine the effects of artificially raised pH. CBA and PCP mineralization experiments were carried as usual on three replicate samples taken from under seven vegetation types. Soils were sampled within a metres radius of particular tree types or directly under smaller plants such as ferns and herbaceous growth. All sites exhibited the same soil profile. pH was raised in the solution applied to the soils to pH 12 by adding 50 μL of 1 M NaOH to the 0.5 ml solution of PCP or CBA added to the soil. Trials showed that the pH of the soils was raised to about 8–8.5 in this manner. Results are summarized in Figure 5, which shows the amount of 3CBA degraded after 32 days (when the curves have leveled off) and the amount of PCP degraded after 58 days. pH treatment had no statistically significant effect on CBA degradation in any of the samples, although in every case the mean mineralization rate was higher in the more alkaline samples. There is no difference in CBA mineralization rates between samples. For PCP,

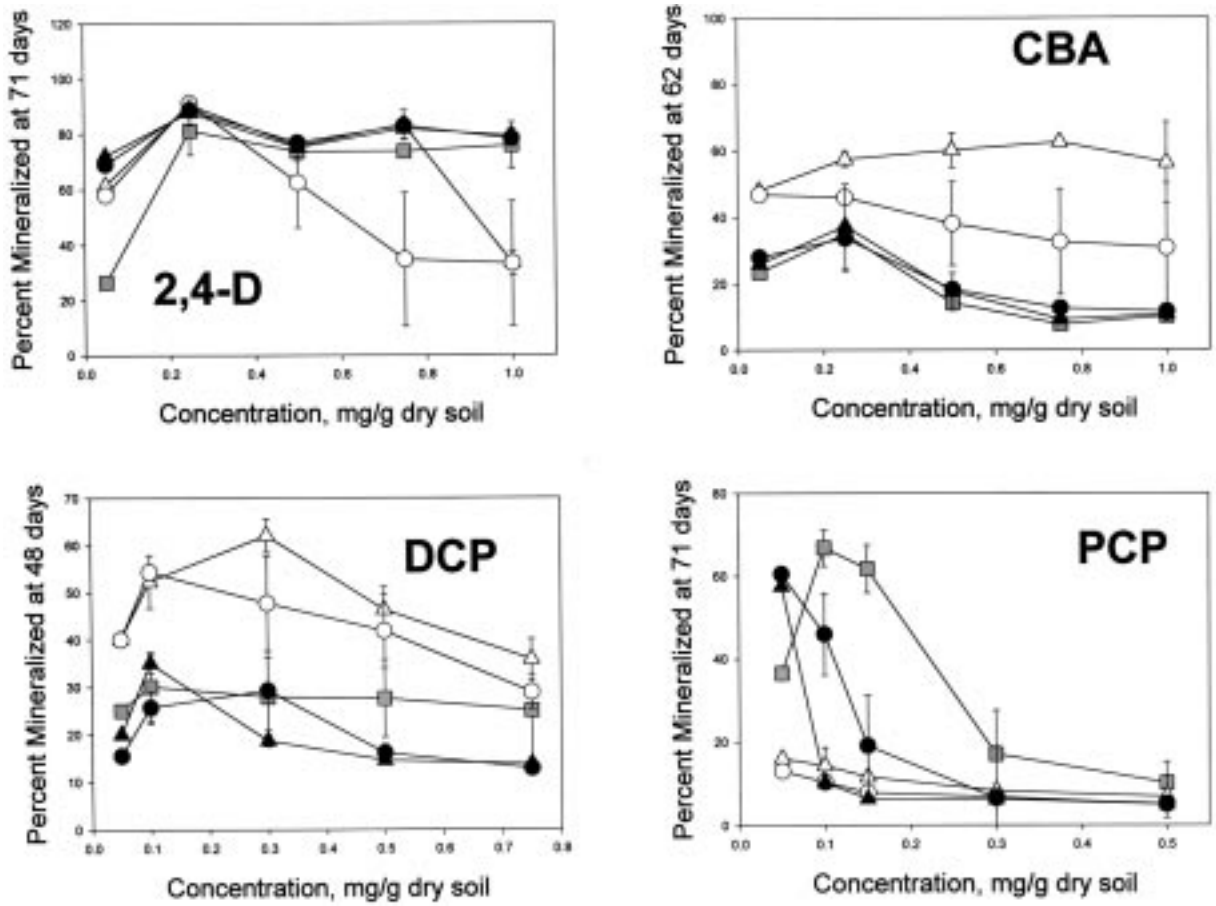

Figure 3. Effect of concentration on the mineralization of a subset of chlorophenolics – averages of cumulative mineralization are shown. Data at 50 μg/g are from 12 samples, data at higher concentrations are from three samples. Bars are standard errors. Open circles = Graythorpe; open triangles = Springwater Pond; filled square = Highland Creek Ravine; black circle = Elora; black triangle = Arkell.

pH has no significant effect, but there are significant differences between samples. When all the replicates are regressed against soil pH, organic content, or moisture content, no significant correlations were found, suggesting that abiotic factors are not responsible for these site differences.

Discussion

This work clearly demonstrates that all soils do not have the same capacity to degrade anthropogenic chemicals. This is particularly important for the modeling of chemical fates in the environment. It is also important to the study of the evolution of biodegradative pathways. In the past, contaminated systems or soils from unspecified systems have been used for enrichment experiments that lead to the isolation of chloroaromatic degraders. It is likely that we are failing to detect the wider diversity of organisms and genetic solutions to the problem of chloroaromatic contamination that probably exists because of our relatively narrow sampling habits. For instance, Kamagata et al. (1997) have shown that 2,4-D degraders isolated from uncontaminated soils collected from various locations are alpha-proteobacteria closely related to *Bradyrhizobium*, in contrast to the more typical *Ralstonia-Burkholderia* and *Sphingomonas* species isolated from agricultural soils.

Our results suggest no selection for organisms able to degrade atrazine or its analogues in forest soils, while atrazine spraying has resulted in its mineralization in agricultural soils. Our data are in agreement with a study by Barriuso and Houot (1996), where less than 4% mineralization of atrazine was observed in unexposed plots. The low and constant

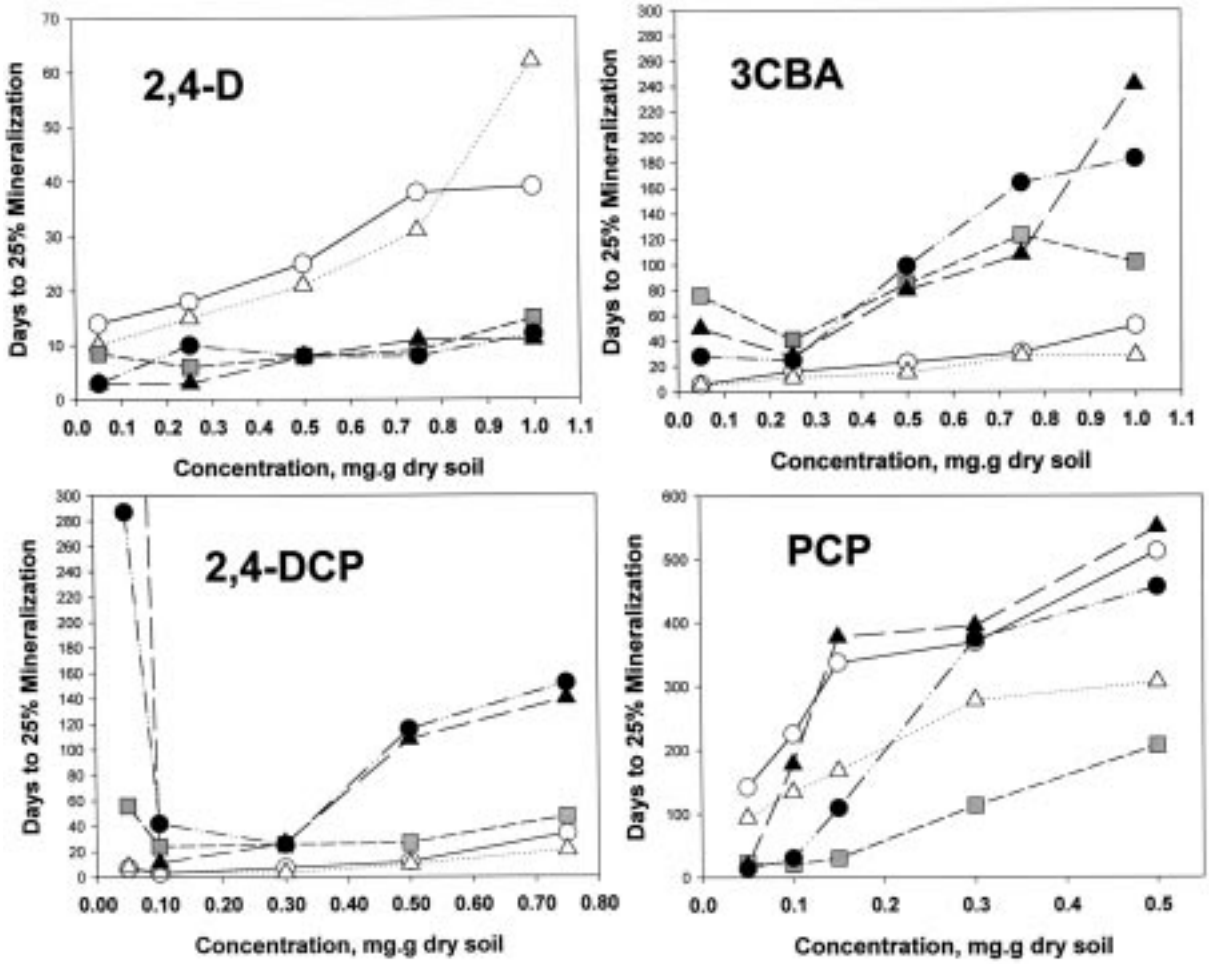

Figure 4. Effect of concentration on the time taken to reach 25% mineralization for a subset of chemicals. Data at 50 μg/g are from 12 samples, data at higher concentrations are from three samples. Open circles = Graythorpe; open triangles = Springwater Pond; filled square = Highland Creek Ravine; black circle = Elora; black triangle = Arkell.

mineralization rate observed in the forest soils is likely caused by the absence of specific microorganisms able to use the atrazine ring as a growth substrate, rather than unfavorable conditions in the soil. Similarly the high rates of mineralization of PCP seen in the agricultural soils could be explained by the use of the herbicide bromoxynil (3,5-dibromo-4-hydroxybenzonitrile). *Sphingomonas chlorophenolica* (previously *Flavobacterium*) sp. strain ATCC 39723 metabolizes PCP and bromoxynil, likely using the same enzymatic pathway; hence, some PCP degraders may be selected for by this herbicide (Topp et al. 1992).

The PCP-mineralization capability of some samples from the forested systems is a new finding. The Graythorpe and Springwater soils that were generally poor at degrading PCP were slightly acidic. The significant correlation between PCP mineralization and pH led us to wonder if the mineralization rates were mostly determined by PCP availability and/or toxicity. PCP adsorbs onto soil more strongly under acid conditions, when it is protonated, more hydrophobic, and less available to microorganisms (McAllister et al. 1996). Similarly the toxicity of PCP increases at low pH because of an increase in the concentration of the undissociated form (Stanlake and Finn 1982). However, the experiments performed with increased pH show that the site where the soil was sampled has a much more dramatic effect than does the pH amendment. Further studies are underway on those sites exhibiting good PCP degradation.

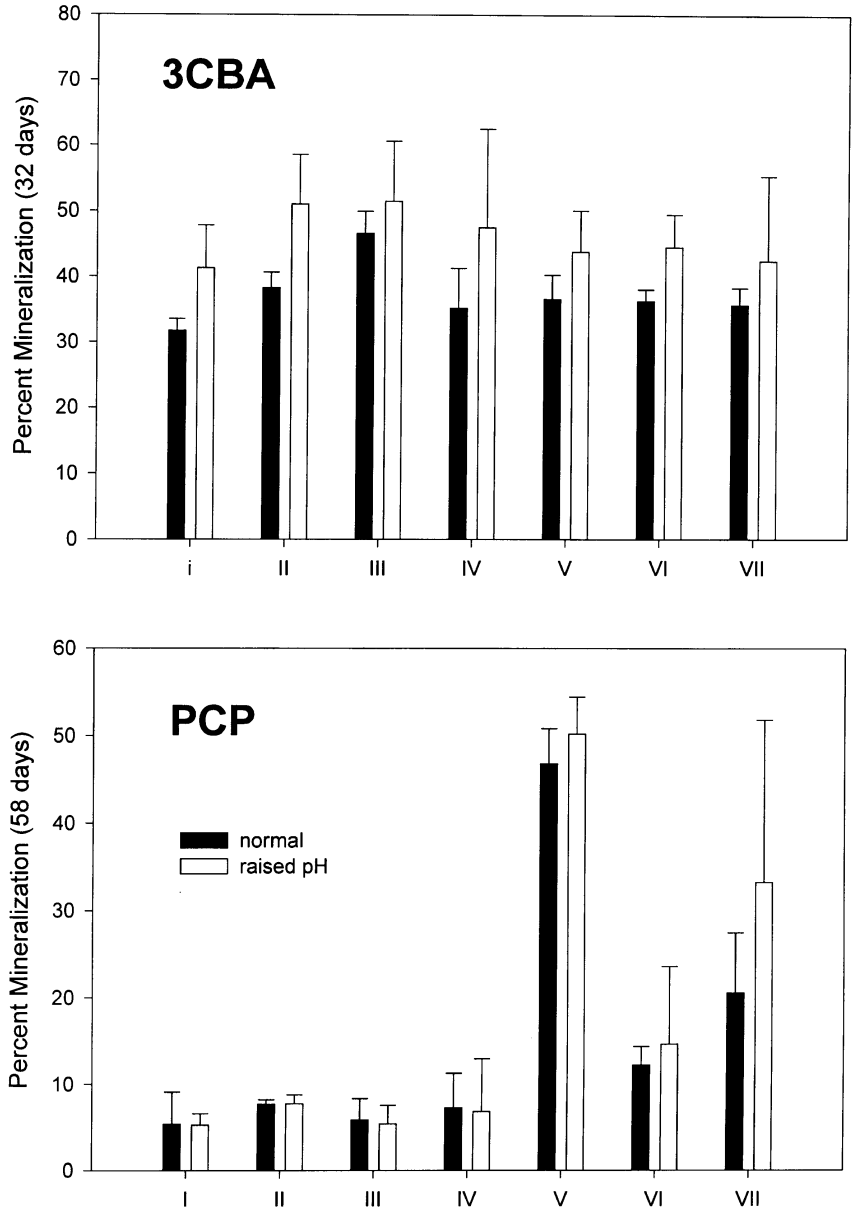

Figure 5. Effect of increased pH on mineralization of CBA and PCP in triplicate samples of soils taken under vegetation dominated by: I = lichen/bryophyes; II = White pine; III = Hemlock; IV = Bunchberry; V = Cedar/Beech Ferns; VI = Lady Ferns; VII = Ephemeral creek bed. Some pH effect is noticeable, but for PCP is outweighed by the effect of the specific sampling site. Bars are standard deviations, on averages from three samples from each vegetation type. Data for CBA are from day 32 when curves had reached maximal cumulative mineralization, and for PCP are from 58 days.

Another new finding of this study is the differences between the soils with respect to DCP degradation. This compound can be naturally occuring in uncontaminated systems (Gribble 1994) and clearly degraders of this compound exist in forested ecosystems. However the ability of the agricultural soils to mineralize DCP was limited and extremely sensitive to high concentrations. This finding is curious given the high 2,4-D mineralization rates of the agricultural soils and the fact that most, if not all, known 2,4-D degraders metabolize this herbicide via DCP (Häggblom 1990). This result might suggest that uptake of

DCP is problematic for agricultural 2,4-D degraders, and/or that the chemicals are processed by different populations in these soils. Similarly, most known 2,4-D degraders are also able to metabolize 3CBA, often with the same enzymes. However, again 3CBA is relatively poorly degraded by agricultural soils, especially at concentrations above 500 μg/g dry soil, in spite of the healthy populations of 2,4-D degraders. The fact that the two systems behave in completely opposite ways with respect to these chemicals underscores the caution of extrapolating the laboratory behaviour of isolated degraders to the real world.

Overall, when the forest soils are compared to the agricultural soils, they exhibit higher mineralizaiton and tolerance of 3CBA and DCP, roughly equivalent mineralization levels of 4-CP and 2,4-D and, at some sites, equivalent PCP mineralization rates. The selection pressure for the evolution of degraders of all five of the chlorophenols in uncontaminated systems is still unknown. The absence of a mixed vegetation on the agricultural soils may explain their overall lower versatility. Conventional cultivation practices are known to decrease the mass and diversity of carbon in soil (Schulten et al. 1995). It has also been found that genetic diversity of microorganisms increases with greater habitat variability (McArthur et al. 1988). The greater amount and diversity of organic matter in forest soil could explain the greater catabolic diversity of microorganisms in forest soils compared to cultivated soils.

The use of forest systems as buffer strips has been proposed by Entry and Ebbingham 1995. They noted improved 2,4-D and atrazine degradation in coniferous forest soils relative to deciduous forest or grassland soils. The results obtained in this study provide strong support for this idea. We have found that rural forested ecosystems are able to mineralize a number of chlorophenolics well and in some cases better than contaminated systems. The capabilities of the Highland Creek Ravine forest seem are a blend of exposed and uncontaminated soils, and show a superior ability than either soil type to degrade PCP, but a decreased ability to utilize CBA and DCP. If forested buffer strips are to be advocated, we must learn about how such buffer strips change in response to greater levels of exposure to the anthropogenic chemicals.

Conclusions

Preexposure of soils to atrazine is required before mineralization is likely to be significant. No preexposure is required for mineralization of 2,4-D, 4CP, DCP, 3CBA, or PCP to take place, although exposure to a suite of herbicides including 2,4-D and bromoxynil enhances 2,4-D and PCP mineralization. Exposure to the same suite of herbicides seems to impair the ability of soils to mineralize 3CBA and DCP. Close proximity to urban development seems to enhance 2,4-D and PCP mineralization, but impairs 3CBA degradation in forest soils. PCP mineralization in uncontaminated forest soils is site specific.

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References

Anderson RA & Coates JR (1995) Screening rhizosphere soil samples for the ability to mineralize elevated concentrations of atrazine and metalochlor. J. Environ. Sci. Health B30: 473–484

Boyle JJ & Shann JR (1995) Biodegradation of phenol, DCP, 2,4–D and 2,4,5-T in field-collected rhizosphere and nonrhizosphere soils. J. Env. Quality 24: 782–785

Brunsbach FR & Reineke W (1993) Degradation of chlorobenzoates in soil slurry by special organisms. Appl. Microbiol. Biotech. 39: 117–122.

De Jong E, Field JA, Spinnler H-E, Wijnberg JBPA & de Bont JAM (1994) Significant biogenesis of chlorinated aromatics by fungi in natural environments. Appl. Environ. Microbiol. 60: 264–270

Entry JA & Emmingham WA (1995) Influence of vegetation of microbial degradation of atrazine and 2,4-dichlorophenoxyacetic acid in riparian soils. Can. J. Soil Sci. 76: 101–106

Focht DD, Searles DB & Koh SC (1996) Genetic exchange in soil between introduced chlorobenzoate degraders and indigenous biphenyl degraders. Appl. Environ. Microbiol. 62: 3910–3913

Fulthorpe RR, Rhodes AN & Tiedje JM (1996) Pristine soils mineralize 3-chlorobenzoate and 2,4-dichlorophenoxyacetate via different microbial populations. Appl. Environ. Microbiol. 62: 1159–1166

Fulthorpe RR, Rhodes AN & Tiedje JM (1998) High levels of endemicity apparent in 3-chlorobenzoate degrading soil bacteria. Appl. Environ. Microbiol. 64: 1620–1627

Gribble GW (1994) The natural production of chlorinated compounds. Env. Sci. Technol. 28: 311A–319A

Häggblom M (1990) Mechanisms of bacterial degradation and transformation of chlorinated monoaromatic compounds. J. Basic Microbiol. 30(2): 115–141

Hernandez BS, Arensdorf JJ & Focht DD (1995) Catabolic characteristics of biphenyl-utilizing isolates which cometabolize PCBs. Biodegradation 6: 75–82

Hickey WJ & Focht DD (1990) Degradation of mono-, di-, and trihalogenated benzoic acids by *Pseudomonas aeruginosa* JB2. Appl. Environ. Microbiol. 56: 3842–3850

Hickey WJ, Searles DB & Focht DD (1993) Enhanced mineralization of polychlorinated biphenyls in soil inoculated with chlorobenzoate-degrading bacteria. Appl. Environ. Microbiol. 59: 1194–1200

Kamagata Y, Fulthorpe RR, Tamura K, Takami H, Forney L & Tiedje JM (1997) Pristine environments harbor a new group of oligotrophic 2,4-dichlorophenoxyacetic acid-degrading bacteria. Appl. Environ. Microbiol. 63: 2266–2272

McAllister KA, Lee H & Trevors JT (1996) Microbial degradation of pentachlorophenol. Biodegradation 7: 1–40

McArthur JV, Kovacic DA &. Smith MH (1988) Genetic diversity in natural populations of a soil bacterium across a landscape gradient. Proc. Nat. Acad. Sci. USA 85: 9621–9624

Sandman ERIC & Loos MA (1984) Enumeration of 2,4-D degrading microorganism in soils and crop plant rhizospheres using indicator media; high populations associated with sugarcane (*Saccharum officianarum*). Chemosphere 13: 1073–1084

Schmidt SK & Scow KM (1997) Use of soil bioreactors and microcosms in bioremediation research. In: Hurst CJ, Knudsen GR, McInerney MJ, Stetzenbach LC & Walter MV (Eds) Manual of Environmental Microbiology (pp. 822–829). American Society for Microbiology, Washington D.C.

Schulten HR, Monreal CM & Schnitzer M (1995) Effect of long term cultivation on the chemical structure of soil organic matter. Naturwissenschaften 82: 42–44

Siciliano SD & Germida JJ (1997) Bacterial inoculants of forage grasses that enhance degradation of 2-chlorobenzoic acid in soil. Environ. Toxicol. Chem. 16: 1098–1104

Staley JT & Konopka A (1985) Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Ann. Rev. Microbiol. 39: 321–346

Stanlake & Finn (1982) Isolating and characterization of a pentachlorophenol-degrading bacterium. Appl. Environ. Microbiol. 44(6): 1422–1427

Topp E, Xun L & Orser CS (1992) Biodegradation of the herbicide bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) by purified pentachlorophenol hydroxylase and whole cells of Flavobacterium sp. strain ATCC 39723 is accompanied by cyanogenesis. Appl. Environ. Microbiol. 58: 502–506