Bioremediation of soils contaminated with organic compounds

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Abstract
Organic contaminants in soil are subject to a number of fate processes. Soil microorganisms can effectively degrade PCP however the fate of dieldrin requires further elucidation. Some organic contaminants are mobile and may leach over time. Plants may degrade organic contaminants, but may also prevent leaching through their water use. Water use may result in plant uptake of soluble contaminants, or simply prevent their movement from the site, thereby reducing off-site effects of contaminants and their dissipation into surrounding environments.

This paper reports the results from a greenhouse –based plant trial assessing the potential of phytoremediation to treat PCP and dieldrin contaminated soils. Changes in PCP and dieldrin soil concentrations, microbial activity, and microbial population structure under various vegetation regimes, were monitored over time. Soil column experiments were also conducted on PCP contaminated soil to investigate the leaching of aged PCP residues and associated contaminants including heavy metals from PCP contaminated soil.

Introduction
The wealth of New Zealand depends on the quality and productive capacity of our soils and environment. A legacy of past, poor environmental practices has created many thousands of contaminated terrestrial sites. Many of these sites contain leachates that are compromising the quality of receiving waters.

Bioremediation is a technology that offers the promise of cost-effective remediation technology. While bioremediation processes often require longer time to effect site improvement they provide an appealing 'green' alternative for cleaning up contaminated sites.

Pentachlorophenol (PCP) and dieldrin are persistent organic pollutants (POPs) with a history of wide spread use in New Zealand (NZ). These POPs pose a threat to the environment, and the health of humans and animals (Orris *et al.* 2000). PCP was used in NZ as a wood preservative until 1986 when its use was banned but many contaminated timber treatment sites still remain today. Dieldrin was used in NZ until the 1960s to control ectoparasites on sheep and many farms now contain contaminated sheep dipping areas. The high number of small, contaminated sites, left as a legacy of these past practices, makes more conventional remediation technologies such as capping, chemical leaching or soil disposal prohibitively expensive. A lower-cost, *in situ* solution is required.

Phytoremediation, the use of plants to remove, contain or render harmless, contaminants from soil or water, is a proven technique for remediating heavy metals. However, less is known about the ability of phytoremediation to treat soils contaminated with POPs.

Here we present data from pot trials where soil, historically contaminated with either dieldrin or PCP, was planted and managed. The aim of this study was to estimate the potential use of phytoremediation to stimulate microbial degradation of PCP and dieldrin.

Materials and Methods
Two parallel experiments were set up in a shadehouse in Palmerston North, New Zealand. Five pots each of grass, *Hebe speciosa*, poplar and willow were planted in 15 L pots containing soil historically contaminated with dieldrin at an average concentration of 64 mg kg^{-1}. PCP contaminated material, collected from a timber treatment yard, was mixed with topsoil to give a concentration of 250 mg kg^{-1}. This concentration was previously assessed as being the upper tolerance limit of poplar and willow to PCP contaminated soil. Four each of poplar and willow were planted in the PCP contaminated soil. In

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both pot experiments the control treatments were unplanted contaminated soils in 15 L pots that received all other soil amendments such as irrigation and fertiliser along with planted replicates.

All pots were placed upon leachate collection trays to prevent any contaminants leaving the pot. The leachate retrieved from the bottom of each pot was reapplied to the soil surface. Dieldrin contaminated soil showed no indications that re-irrigation of leachate was damaging to either the plant or the soil. However PCP contaminated soil appeared to become water logged and poorly drained following repeated application of leachate. We suspect that these soil characteristics have developed due to other co-contaminants present in the original PCP contaminated material altering the characteristics of the soil within the pots. In order to improve our understanding of the changes to soil contaminated with PCP further investigations using column leaching apparatus were performed on soils contaminated with PCP at either 250 or 600 mg kg^{-1} (Figure 1).

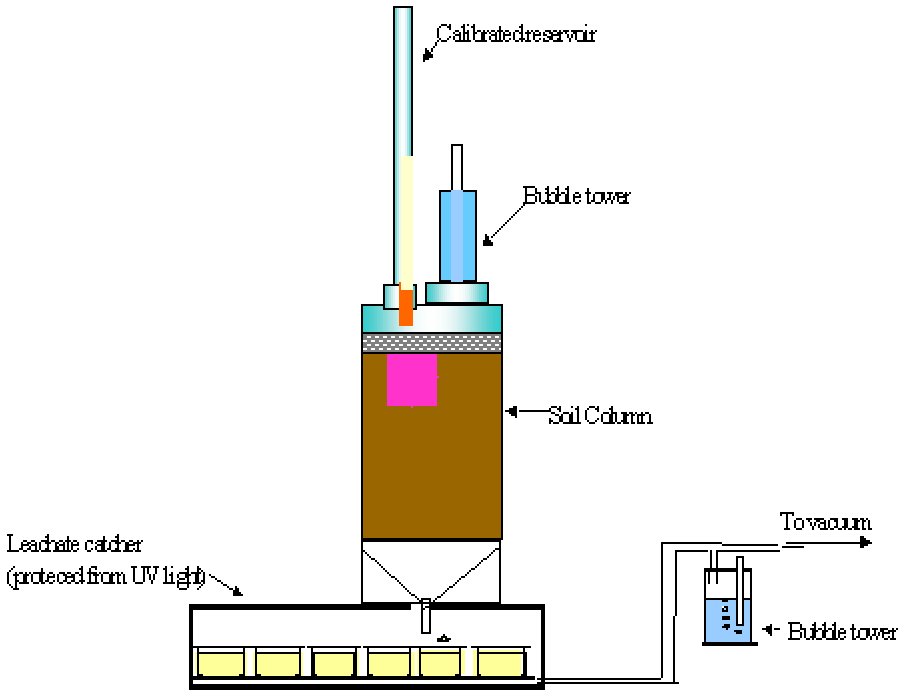

Figure 1. Column leaching apparatus - for the investigation of PCP and co-contaminant concentrations in leachate.

Indigenous soil microbial populations are responsible for the degradation of organic contaminants in soil. Both number and type of microorganisms influences the efficacy of microbial degradation. Soil samples from both dieldrin and PCP pots were tested monthly for soil microbial activity using a dehydrogenase assay (triphenylformazan = TPF) (Chander and Brookes 1991). Soil microbial numbers were calculated monthly using serial dilutions and plating. At Day of Experiment (DOE) 600 only grass, *Hebe speciosa*, poplar and willow from each experiment were carefully removed from the soil and the roots analysed under a microscope for mycorrhizal fungal colonies

Results and Discussion

Microbial Activity and degradation

Soil microbial activity as measured using a dehydrogenase assay, indicates that plants significantly increased the level of microbial activity in both PCP (Figure 2) and dieldrin contaminated soil (data not shown). Although plants stimulate microbial activity there were no consistent changes in the population of microbes present in the soil of either experiment given differing vegetation types during the first 300 DOE. We did note however that the number of fungi present in soil following contamination with PCP material dropped significantly. We expect this, as PCP is a known fungicide.

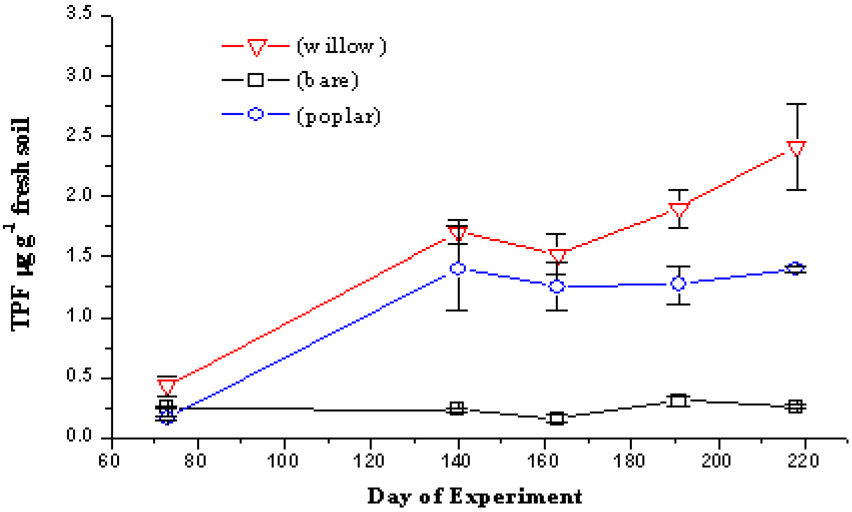

Figure 2. Dehydrogenase activity as indicated by the transformation of triphenyltetra zolium chloride (TTC) to triphenylformazan (TPF) for PCP contaminated soil planted with either poplar, willow or left unplanted. Error bars represent standard errors of the mean.

When root tissue was studied at DOE 600 under a microscope we observed developed mycorrhizal fungal colonies on the plant roots growing in soil previously contaminated with PCP (Figure 3). In contrast very few mycorrhizal fungal colonies are identified on the roots of plants from the dieldrin-contaminated soil. (Figure 4).

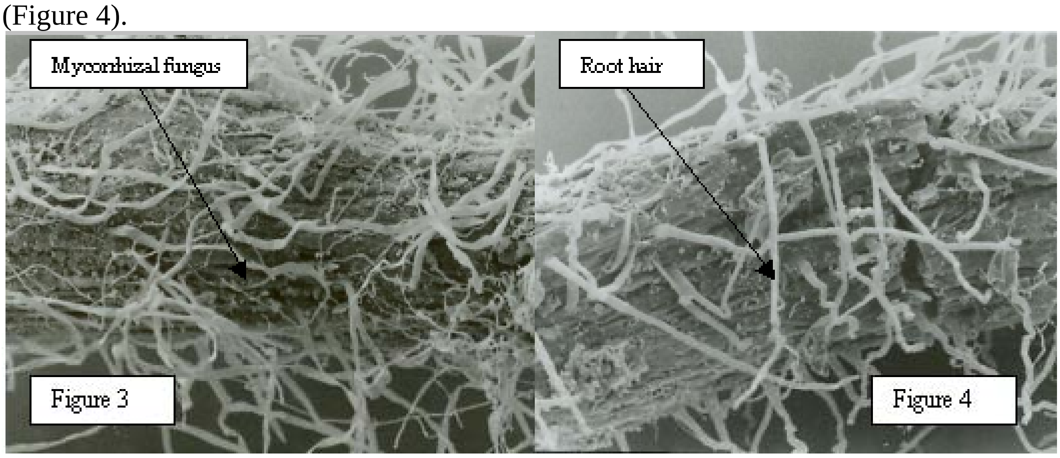

Figure 3. Scanning electron microscopy x200 (SEM), willow root hairs growing in soil previously contaminated with PCP. Figure 4. SEM, willow root hairs growing in soil previously contaminated with dieldrin.

The measured increase in microbial activity in all planted soil in both experiments did not always correlate with stimulated degradation of contaminants. Consistent reductions in measured levels of PCP were recorded during the potted experiment (Figure 5) in contrast to variable results obtained for dieldrin degradation (Figure 6).

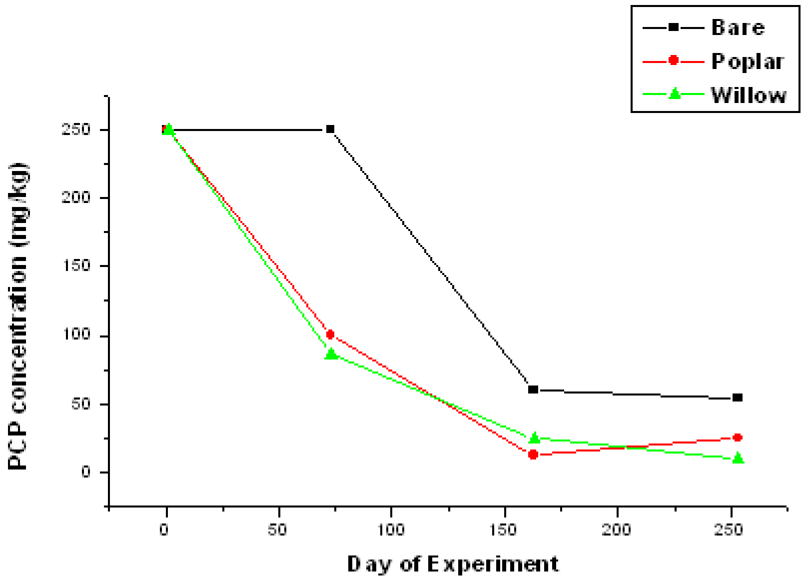

Figure 5. Change in PCP concentration from initial concentration of 250 mg kg^{-1} with time in pots planted with either poplar, willow or left unplanted.

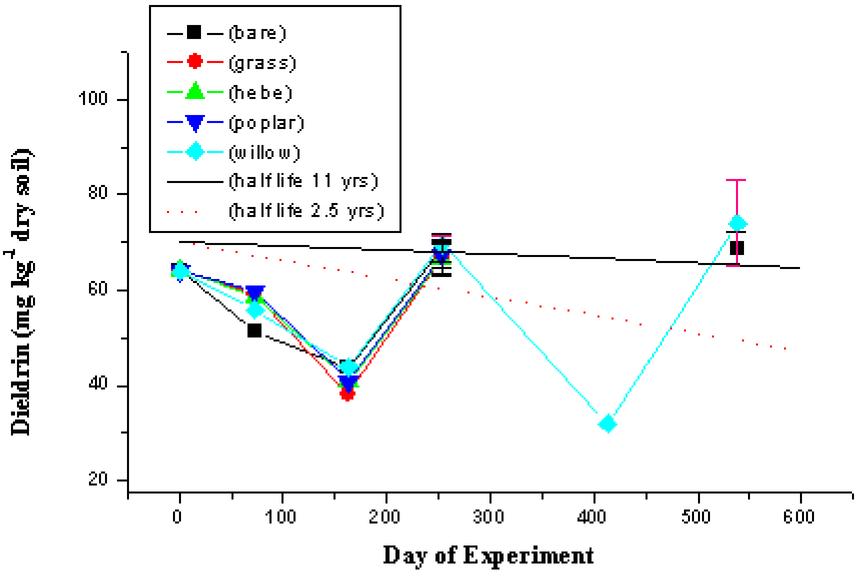

Figure 6. Change in dieldrin concentration over time for planted and unplanted (bare) soil and estimated degradation assuming published half-life values for dieldrin in soil (Brown (1978), State of the Environment Report (1997, pg 8.67)).

The data show that planted treatments produced a higher rate of initial PCP loss but by day 160 the difference between PCP levels in the planted and non-planted treatments is reduced. Therefore, the planted treatments provide a higher degradation rate initially, but give only a limited improvement in the degradation endpoint. Plants encourage accelerated degradation of PCP however dieldrin degradation is poorly understood. We see fluctuating levels of total dieldrin in our sample during the experiment. This suggests changes in the partitioning of dieldrin within the soil matrix. Without performing a sequential extraction it is difficult to gauge how much of the dieldrin is recorded at any sampling time.

Although PCP is readily degraded under phytoremediation the requirement to prevent PCP migrating from the site may require a capture and return system. Table 1 gives values of PCP captured in leachate leaving the contaminated soil columns. The presence of PCP and other co-contaminants in leachate exiting PCP contaminated soil may pose an environmental threat.

Column Leaching Experiment (PCP)

Column leaching experiments showed reduced flowrate through the PCP contaminated soil column at both 250 and 600 mg kg^{-1} PCP (Figure 7). The leachate collected from the columns contained significant levels of B with traces of Cu, Cr and As (Table 1). All four of these metals were also detected in the soil packed into the column (data not shown).

The dilution of contaminated material with topsoil was necessary to establish plants and healthy microbial populations in our experimental pots. This does mean however that both heavy metal and PCP concentrations within the experimental soil are significantly reduced from those typically present at contaminated sites.

The observed reduction in flow rate in the packed soil columns corresponded with the appearance of an oily film on the collected leachate. Analysis of soil used to pack the column and column leachate demonstrated the absence of Polycyclic Aromatic Hydrocarbons (PAH's) and other components found in creosote. We analysed soil and leachate for these co-contaminants as creosote was commonly used as the carrier solution for PCP timber treatment in NZ. The source and composition of the components responsible for the oily film is unknown but may include light oils or diesel residues that were not tested for.

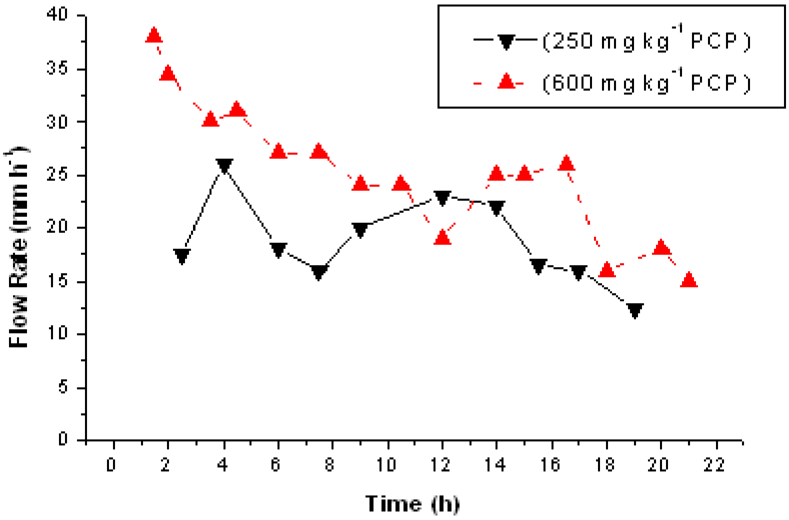

Figure 7. Change in flow-rate of water and leachate through soil columns contaminated with either 250 mg kg^{-1} PCP or 600 mg kg^{-1} PCP in soil.

Table 1. Concentrations of metals in the leachate from columns contaminated with either 250 or 600 mg kg^{-1}PCP contaminated material.

Sample	Boron (B) mg l^{-1}	Copper (Cu) mg l^{-1}	Chromium (Cr) mg l^{-1}	Arsenic (As) mg l^{-1}	PCP (mg l^{-1})	TCP*** (mg l^{-1})
Leaching event 1*	0.83	0.05	0.01	0.02	13.1	0.8
Leaching event 2*	0.40	0.03	0.01	0.01	1.6	Below detection
Leaching event 3*	0.78	0.02	0.01	0.02	1.1	Below detection
Leaching event 1**	2.86	0.07	0.03	0.01	0.5	Below detection
Leaching event 2**	1.64	0.05	0.03	0.01	7.0	0.5
Leaching event 3**	2.62	0.06	0.03	0.01	6.1	0.5

* (250 mg kg^{-1} PCP)
** (600 mg kg^{-1} PCP)
*** Tetrachlorophenol – degradation product of PCP

Conclusions

- Plants stimulate microbial degradation but this does not necessarily parallel reductions in organic contaminant concentrations
- Dieldrin degradation requires further elucidation
- Successful phytoremediation of soil containing 250 mg kg^{-1} PCP is demonstrated
- Co-contaminants at timber treatment sites (As, Cr, Cu and B) may limit plant growth and reduce microbial activity
- In addition to reducing the concentration of site contaminants plants also offer the advantage of altering the water balance of a site, thereby reducing contaminant leaching and off-site movement.

Acknowledgements

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