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Changes in Cd and Cr fluxes during the bioremediation of phenanthrene

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Abstract. Different types of contaminants such as polycyclic aromatic hydrocarbons (PAHs) and metals coexist in different chemical forms in soil. Although bioremediation of organics has been widely studied, little attention has been paid to changes in metal behaviour during the bioremediation process. In this study, the diffusion gradient in thin-film (DGT) technique was used to assess changes in potentially bioavailable Cd and Cr fluxes in soil during the bioremediation of phenanthrene. Fluxes of Cd were low ($<0.2\,\mathrm{pg\,cm^{-2}\,s^{-1}}$) and increased to $0.4–0.9\,\mathrm{pg\,cm^{-2}\,s^{-1}}$, while Cr(III) fluxes showed an approximately tenfold increase. Results showed that there was increased mobilization of metals associated with the fungal reduction of phenanthrene concentrations by *Penicillium frequentans.*

Keywords: Cadmium, chromium, bioavailability, DGT, fluxes

INTRODUCTION

Remediation of polycyclic hydrocarbons (PAHs) in soil has received much attention in recent years. However, different types of contaminants such as PAHs and metals coexist in different forms in soil. Phenanthrene is one of the most common PAHs and is a priority pollutant (WHO 2001). The fungus *Penicillium frequentans* has recently been shown to effectively remove phenanthrene in soil (Amezcua-Allieri *et al.* 2003). In addition, we have shown that use of the fungal remediation method leads to significant increase in the flux of certain metals (Cu, Pb, Ni) from solid phase to solution phase (Amezcua-Allieri *et al.* 2005). Nevertheless, the effect of this process on metal speciation and thus behaviour is poorly understood.

In this study, changes in metal fluxes of both cadmium (Cd) and chromium (Cr) were quantified using the diffusion gradient in thin-film (DGT) technique. DGT performs an *in-situ* speciation measurement, with only the smallest, most labile forms of the solution phase taken up by the DGT device. Specifically, it has been shown that only one oxidation state of chromium (Cr(III)) is bound to the resin layer and analysed (Ernstberger *et al.* 2002). The metals Cd and Cr were chosen because they are significant contaminants in soils, with potential effects on soil and plant health. Although the concentrations are relatively low, interest pertains to changes in fluxes and therefore changes in potentially bioavailable metals, rather than the total levels.

MATERIALS AND METHODS

Previously, the chosen soil was fully characterized (Amezcua-Allieri *et al.* 2003). In brief, pH is 5.7 ± 0.1; organic matter, 6.8% (total organic carbon 4.1%); N, 0.3%; and cation exchange capacity (CEC) $5.1\,\mathrm{meq}\,100\,\mathrm{g^{-1}}$. Concentrations of phenanthrene were below detection limits ($0.002\,\mathrm{mg\,kg^{-1}}$) prior to spiking. Total Cd and Cr concentrations were $18\,\mathrm{mg\,kg^{-1}}$ and $23\,\mathrm{mg\,kg^{-1}}$, respectively.

The propagation of *P. frequentans* was performed over 7 days in the dark at 26 °C in PDA media. In order to obtain a fungal mycelium in pellets, fungus was grown in modified liquid Wunder media (Wunder *et al.* 1994) over 4 days. Soil suspensions were kept under constant conditions over the 30-day period of the experiment as previously optimized (Amezcua-Allieri *et al.* 2003). On day 14, 12.0 g of non-sterilized soil (including soil native microflora) was separately spiked with HPLC-grade phenanthrene in acetone 24 hours before inoculation, in order to let the solvent evaporate. The final phenanthrene concentration was $200\,\mathrm{mg\,kg^{-1}}$. On day 15 the spiked soil was inoculated with the fungus in pellets under conditions previously optimized (Amezcua-Allieri *et al.* 2003). Treatment 1 contained soil (S) + fungus (F) + phenanthrene (P); treatment 2, S + F; treatment 3, S + P; and treatment 4, S only. Treatments 3 and 4 were controls to calculate the net impact of the fungus. All treatments contained growth media. We have previously shown (Amezcua-Allieri *et al.* 2003) that the fungus is successful in removing phenanthrene from soil.

Metal fluxes were studied using DGT, which separates species kinetically and does not rely on the establishment of equilibrium. Solutes freely diffuse through a layer of hydrogel and are then immobilized in an underlying layer of Chelex. DGT devices were deployed in the soils for a maximum of 30 days and removed (in triplicate) every 3

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days (day 3, day 6, etc.). Metals were eluted from the Chelex by 1 M HNO_3 and then measured using Varian M493 graphite furnace atomic absorption spectroscopy, allowing the Cd and Cr fluxes to be calculated (Davison *et al.* 2000a). Metal analysis was performed in triplicate.

RESULTS

Figure 1 shows the changes Cd and Cr(III) fluxes as measured by DGT (Ernstberger *et al.* 2002) before and after fungal inoculation on day 15. Prior to fungal addition, Cd fluxes were low ($<0.2\,\mathrm{pg\,cm^{-2}\,s^{-1}}$) and increased to $0.4–0.9\,\mathrm{pg\,cm^{-2}\,s^{-1}}$. Significant differences were found ($P < 0.005$) between treatments 1 and 2 (fungus present) and treatments 3 and 4 (fungus absent). In addition, treatment 1 (phenanthrene present) was significantly ($P < 0.005$) higher than treatment 2 (phenanthrene absent).

Prior to fungal addition, Cr(III) fluxes were low ($<0.04\,\mathrm{pg\,cm^{-2}\,s^{-1}}$) and increased to $0.3–0.4\,\mathrm{pg\,cm^{-2}\,s^{-1}}$, an approximately tenfold increase. Significant differences were found ($P < 0.003$) between treatments 1 and 2 (fungus present) and treatments 3 and 4 (fungus absent) as with Cd. In addition, treatment 1 was significantly ($P < 0.002$) higher than treatment 2.

Soil pH values in controls and treatments 1 and 2 were measured before and after 30 days and in all cases varied by only small amounts. Values at the start of the experiment were 5.7 ± 0.1 and after 30 days were 5.4 ± 0.1 for all controls and treatments. No significant differences were found between the treatments with fungi and the controls without fungi.

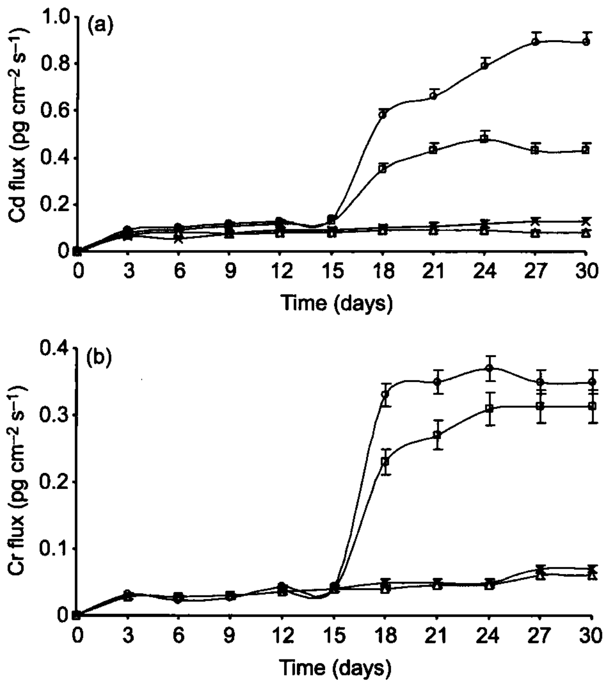

Figure 1. Fluxes measured by DGT for (a) cadmium and (b) chromium. Treatment 1 (o): soil + fungus + phenanthrene; treatment 2 (□): soil + fungus; treatment 3 (Δ): soil + phenanthrene; treatment 4 (X): soil only. On day 15, *P. frequentans* was added to the soil.

DISCUSSION

Previously, we have shown that phenanthrene concentrations are reduced by the action of the fungi over the period of this study, that is, bioremediation has occurred (Amezcua-Allieri *et al.* 2003; Amezcua-Allieri *et al.* 2005). Here we only sought to show that the metal flux as measured by DGT, which is strongly related to the solid–solution transport of metals, is increased as the result of this bioremediation.

It is important to realize what DGT actually measures. The DGT method provides directly a measure of the flux from the solution to the DGT device. As the solution phase will be rapidly depleted by the perturbation caused by the DGT device, further uptake to the DGT depends on a re-supply of the solution phase, either from the solid phase or by diffusive movement from elsewhere in the soil. As diffusion is slow (Davison *et al.* 2000a), the DGT derived flux is a measure of the re-supply of metal from the solid phase.

In addition, DGT has also been shown to be a good surrogate for metal uptake by plants (Davison *et al.* 2000b). Therefore, these results show that the bioremediation process significantly increased the mobilization of Cd and Cr(III) and therefore the potential bioavailability of these metals.

The mechanisms of mobilization are not clear. However, metal mobilization due to bulk pH changes could not have occurred, as sample pH varied by the same small amount (less than 0.3 units) in the presence or absence of fungi. In the absence of fungi, no further metal mobilization was observed (Figure 1). In addition, the bulk solid phase is unlikely to have been affected significantly because of the short timescales involved. It seems most likely that the fungus acted so as to release surface bound metal, perhaps through the release of organic complexing ligands or acids, which changed the pH at the soil surface only; confirmation of this awaits further study, requiring sophisticated microscopy and spectroscopy.

Clearly, the results from this study have important implications for the strategies designed to bioremediate contaminated land. Importantly, these strategies should consider the impact of bioremediation on the whole system (including a range of pollutants). Bioremediation strategies must include mechanisms for the fixation of mobilized pollutants or strictly assess the relative risks of remediation and mobilization. It is insufficient to consider the reduction of a single pollutant or group of pollutants when assessing the feasibility of remediation.

CONCLUSIONS

Bioremediation of phenanthrene in soils resulted in the increased release of toxic metal species. Consideration of these interconnected processes must occur to ensure a suitable use of remediation techniques.

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