Using rhizosphere biogeochemistry to increase understanding of heavy metal bioavailability

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Abstract

A greater understanding of rhizosphere biogeochemistry is likely to improve our understanding of the factors that drive bioavailability of heavy metals in growth substrates. A glasshouse study was conducted that investigated the impacts of three growth substrates (topsoil, oxidised mining waste and unoxidised mining waste) and four species of New Zealand trees on the rhizosphere concentrations of arsenic, copper and zinc. In many of the substrate by species treatments the concentration of heavy metals was lower in the rhizosphere than in the bulk substrate. In addition, for a given plant species, where rhizosphere concentrations were significantly different to the bulk concentration, the rhizosphere concentration correlated better with plant shoot concentrations (i.e. bioavailability) than they bulk concentrations of heavy metals. In particular, the rhizosphere concentrations were good predictors of plant copper concentrations.

Key Words

Rhizosphere, mine rehabilitation, heavy metals, plant nutrition, soil formation.

Introduction

Researchers have been attempting to develop a robust method of assessing heavy metal bioavailability for decades. A number of potential methods have been suggested but as yet, none have been demonstrated to have wide applicability. In response, a growing body of research is now investigating rhizosphere biogeochemistry to increase our knowledge of the factors affecting heavy metal bioavailability and thus the development of robust soil bioavailability tools. While many researchers have investigated the impacts of microbes on heavy metal dynamics in the rhizosphere there have been fewer investigations into the direct impact of plants on rhizosphere biogeochemistry. In addition, most research has focused on soil with little emphasis on other growth substrates such as mining wastes. The following study investigated the effects of a four New Zealand tree species on the rhizosphere concentrations of arsenic, copper and zinc in soil and mining wastes.

Methods

The experiment was conducted as a pot trial in a glass house at Lincoln University, New Zealand. Three growth substrates were trialed, *viz.*, stockpiled topsoil, oxidized mining waste and un-oxidised mining waste (Table 1). All growth substrates were collected from the Globe-Progress Mine, New Zealand. The substrates were air dried and passed through a 1.5 cm sieve before use in the experiment. Plastic pots were lined with a plastic bag and filled with air-dry substrate (2.1 kg topsoil, 2.6 kg oxidised mining waste, 2.5 kg unoxidised mining waste). Fertiliser was added as the dry analytical reagent grade compound at a rate of 150 kg/ha N (as NH_4NO_3) and 50 kg/ha P (as KH_2PO_4) and thoroughly mixed into each pot.

Table 1. Description of topsoil and mining wastes collected from Globe-Progress Gold Mine, New Zealand.

Parameter	Topsoil	Oxidised waste	Un-oxidised waste
pH (1: 5 substrate: H_2O)	4.8	4.4	5.1
Electrical conductivity (1: 5 substrate: H_2O) (μS/m)	100	40	58
Gravimetric water content at field capacity (g g^{-1})	0.31	0.13	0.16
Specific surface area (m^2/g)	0.525	0.563	0.811
Clay (%)	1.9	2.1	3.1
Silt (%)	64.3	54.7	77.9
Sand (%)	33.8	43.2	19.1

Four New Zealand tree species used in mining restoration were trialed, viz. *Nothofagus truncata* (red beech), *Leptospermum scoparium* (manuka), *Aristotelia serrata* (wineberry) and *Griselina littoralis* (broadleaf). Seedlings were collected from an unmined area on the Globe Progress Site, and transported bare-rooted and wrapped in moist sphagnum moss to Lincoln University. One seedling was planted per pot with five replicate pots of each species by substrate combination. Approximately 275 g of white polythene beads (5 mm diameter) were placed on the surface of each pot to minimise evaporation. The pots were watered with deionised water to 90 % field capacity and maintained at 90 % field capacity throughout the experiment by regular watering with deionised water. The pots were placed in a randomised block design to counterbalance environmental variation within the glasshouse. Every four weeks each block was cycled one place through the glasshouse and the pots within a block re-randomised.

On the 105th day after transplant the experiment was harvested. Plant shoots were cut at the surface of the growth substrate and rinsed sequentially in tapwater, 2 % Decon in tapwater and 2 deionised water rinses before placing in paper bags and drying at 60 ºC for 48 h. Plant tissues were ground and digested in 70 % HNO_3 before analysis for As, Cu and Zn by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). Samples of the bulk substrate were collected using a 1 cm diameter auger. Roots were then gently removed from the substrate and rhizosphere substrate was collected using the method of Gobran *et al*. (1998). Growth substrates samples were air dried before analysis. Soluble As, Cu and Zn was determined by extracting the growth substrate in 0.05M $Ca(NO_3)_2$.

Statistical analyses (Paired t-tests and Pearson correlation coefficient) were performed using MINITAB (Minitab 1995). Data was considered significant when $P<0.1$.

Results
The concentrations of arsenic, copper and zinc showed a range of responses when comparing bulk to rhizosphere concentrations (Figure1). In particular, copper concentrations tended to be lower in the rhizosphere for all species and soil concentrations (Figure 1b, e, h, k). For the few species by substrates combinations where the rhizosphere was not significantly different to the bulk concentration of copper, the concentration appeared to be trending lower in the rhizosphere. Arsenic concentrations were the most variable with difference between bulk and rhizosphere concentration varying from bulk>rhizosphere to no significant difference to bulk<rhizosphere (Figure 1a, d, g, j). Rhizosphere zinc concentrations were the least likely to be differentiated from the bulk substrate concentration of the heavy metals tested (Figure 1c, f, I, l). The only substrate where rhizosphere zinc was consistently significant different to bulk concentrations was in the oxidized waste rock where the bulk>rhizosphere. However the trend was bulk>rhizosphere for zinc concentrations in a number of other species by substrate combinations.

The rhizosphere was a better predictor of plant heavy metal concentrations than the bulk substrate concentration, and thus of heavy metal bioavailability, for each species by metal combination where more than one substrate had significant differences between bulk and rhizosphere concentrations and the change in concentration was in the same direction. In particular, rhizosphere concentrations gave consistently better relationships with shoot copper concentrations. For manuka, wineberry and broadleaf the difference between using bulk and rhizosphere copper concentrations as a predictors of bioavailability was the difference between nonsignificant and significant correlations.
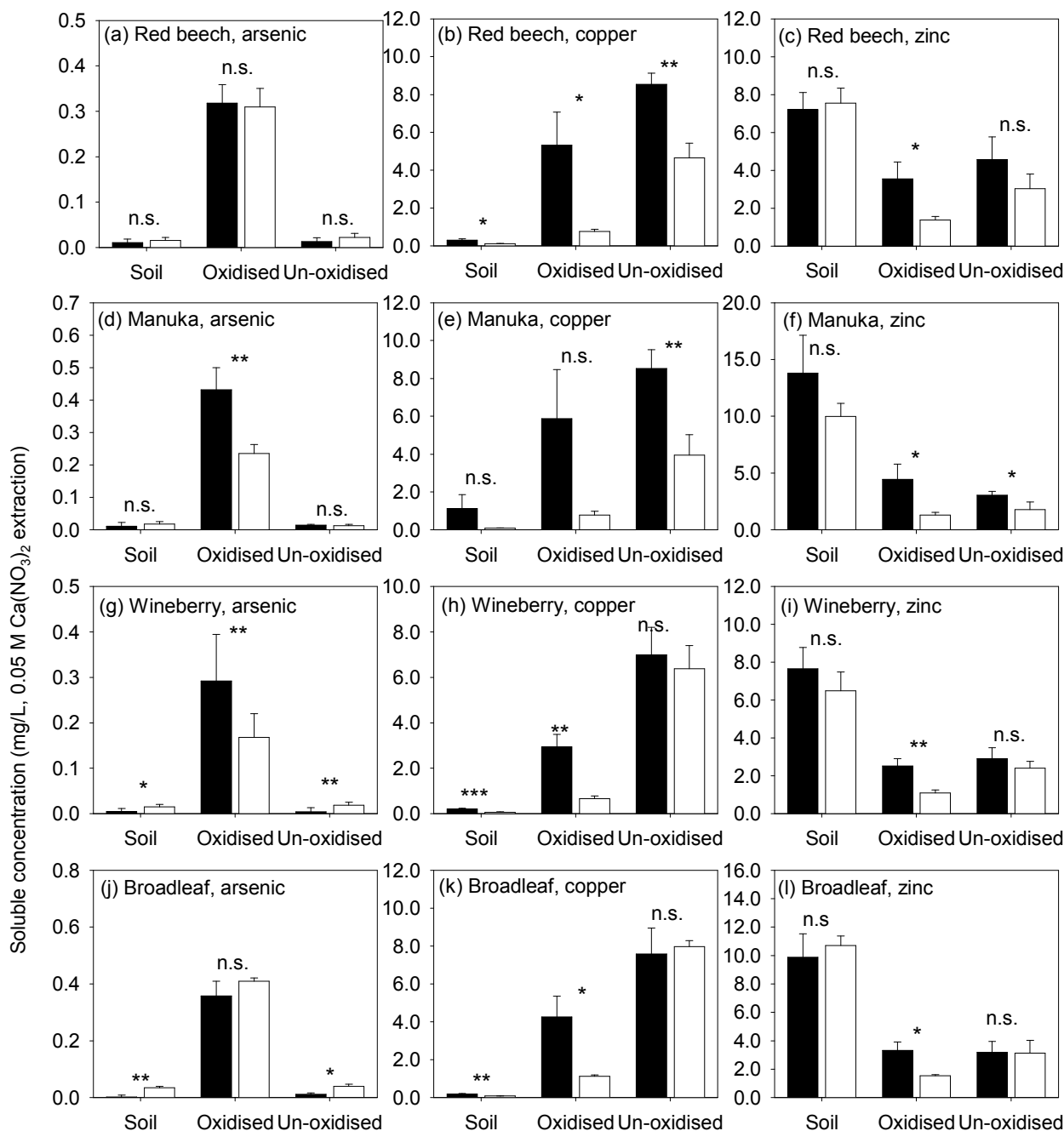
Figure 1. Relationship between the concentration of 0.05 M Ca(NO₃)₂ extractable arsenic, copper and zinc in the bulk substrate (■) and rhizosphere (□) for four New Zealand plant species grown in topsoil and two mining wastes (oxidised and un-oxidised) collected near Reefton, New Zealand. Values above paired columns correspond to the statistical significance of paired t-tests (P<0.01 ***, P<0.05, P<0.1 *, P>0.1 n.s.)**

Table 2. Pearson correlation coefficients between bulk and rhizosphere concentrations for four New Zealand tree species. The plants were grown in three substrates with results combined for this analysis. Shaded squares correspond to treatments where at least two of the substrates had significantly different rhizosphere to the bulk concentrations and the direction of change was consistent between substrates (Figure 1).

Species	Arsenic		Copper		Zinc	
	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere
Red beech	r=0.691	r=0.732	r=0.516	r=0.814	r=0.691	r=0.688
	P=0.009	P=0.004	P=0.071	P=0.001	P=0.009	P=0.009
Manuka	r=0.623	r=0.764	r=0.220	r=0.570	r=0.806	r=0.880
	P=0.017	P=0.001	P=0.450	R=0.033	P<0.001	P<0.001
Wineberry	r=0.774	r=0.654	r=-0.380	r=-0.481	r=0.860	r=0.841
	P=0.001	P=0.008	P=0.163	P=0.070	P<0.001	P<0.001
Broadleaf	r=0.525	r=0.757	r=0.288	r=0.645	r=0.856	r=0.885
	P=0.054	P=0.002	P=0.317	P=0.013	P<0.001	P<0.001

Discussion

In contrast to the current study (Figure 1), rhizosphere concentrations of heavy metals are commonly found to be higher than the corresponding bulk concentration (Hinsinger *et al*. 2009). One reason for the difference between this and other studies may be the prevalence of microbes in different substrates. Most previous studies have used soils that have long histories of plant growth, and thus, are likely to have high concentrations of microbes even in cases where the soil was moderately contaminated with heavy metals. In contrast, the mining wastes used in the current study had no previous history of plant growth and the soil had been stockpiled, and thus, were all likely to have low microbial activity. Therefore, the findings from the current study may be more indicative of direct plant impacts on the rhizosphere such as the rhizosphere representing a zone of depletion from absorbed heavy metals. In addition, the rhizosphere enrichment of arsenic for wineberry and broadleaf in the soil and un-oxidised waste (Figure 1 g, j) may indicate that these species were able to rapidly encourage a microbial population in their rhizosphere in these substrates. Wineberry is a pioneer species in New Zealand ecosystems (Salmon 1996) and thus may have evolved properties to encourage the rapid colonisation of its roots by appropriate microbes. These findings highlight the need to investigate the role of rhizosphere biogeochemistry in a number of substrates and situations to inform our understanding of heavy metal bioavailability.

The research in the current study has illustrated that rhizosphere concentrations of arsenic, copper and zinc are often better predictors of substrate bioavailability than the corresponding bulk concentration. These results confirm the importance of the rhizosphere for our understanding of bioavailability and suggest, in particular, that an understanding of rhizosphere biogeochemistry has a role in a risk-based approach to mining remediation

References
Gobran RG, Clegg S, Courchesne F (1998) Rhizospheric processes influencing the biogeochemistry of forest ecosystems. *Biogeochemistry* **42**, 107-120.
Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. *Plant and Soil* **321**, 117-152.
Minitab (2005) MINITAB Version 14.2. (Minitab Inc: State College, USA)
Salmon JT (1996) The Native Trees of New Zealand. (Reed Books: Auckland).