



Influence of Indian mustard (*Brassica juncea*) on rhizosphere soil solution chemistry in long-term contaminated soils: A rhizobox study

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Abstract

This study investigated the influence of Indian mustard (*Brassica juncea*) root exudation on soil solution properties (pH, dissolved organic carbon (DOC), metal solubility) in the rhizosphere using a rhizobox. Measurement was conducted following the cultivation of Indian mustard in the rhizobox filled four different types of heavy metal contaminated soils (two alkaline soils and two acidic soils). The growth of Indian mustard resulted in a significant increase (by 0.6 pH units) in rhizosphere soil solution pH of acidic soils and only a slight increase (< 0.1 pH units) in alkaline soils. Furthermore, the DOC concentration increased by 17–156 mg/L in the rhizosphere regardless of soil type and the extent of contamination, demonstrating the exudation of DOC from root. Ion chromatographic determination showed a marked increase in the total dissolved organic acids (OAs) in rhizosphere. While root exudates were observed in all soils, the amount of DOC and OAs in soil solution varied considerably amongst different soils, resulting in significant changes to soil solution metals in the rhizosphere. For example, the soil solution Cd, Cu, Pb, and Zn concentrations increased in the rhizosphere of alkaline soils compared to bulk soil following plant cultivation. In contrast, the soluble concentrations of Cd, Pb, and Zn in acidic soils decreased in rhizosphere soil when compared to bulk soils. Besides the influence of pH and DOC on metal solubility, the increase of heavy metal concentration having high stability constant such as Cu and Pb resulted in a release of Cd and Zn from solid phase to liquid phase.

Key words: dissolved organic carbon; heavy metals; root exudates; solubility; rhizosphere chemistry

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Introduction

There are many technologies currently available to remediate contaminated sites. Amongst these phytoremediation has recently emerged as a perceived green alternative to expensive and ecologically instructive conventional engineering-based remediation technologies (Lim et al., 2004). This technology exploits higher plants to remove contaminants from the soil by translocation from soils to plants and has the advantages of being cost-effective, environmentally friendly, and less disruptive to the soil and therefore associated site ecosystem (Salt et al., 1995).

However, in terms of efficiency, phytoremediation still has a number of limitations for practical use including slow growth (low biomass) of hyperaccumulator species and often low metal availability in soils. For these reasons, many studies have been conducted to overcome these limitations via the use of high biomass producing plants associated with chelates such as EDTA (Chen and Cutright, 2001; Jensen et al., 2009). Chelates enhance availability and subsequently increase metal uptake by plants (Epstein et al.,

1999; Lombi et al., 2001; Wenzel et al., 2003). However, it is not clear in the existing studies about the reasons for high metal uptake by accumulator plants. The obvious thought is the fact that even in soils with high binding capacity for metals, hyperaccumulator plants take up more metals than non-accumulators. At present the elevated metal uptake by accumulator plants have been attributed to three mechanisms, including: (1) enhanced adsorption of metals into the roots (Lasat et al., 1996) followed by high rates of translocation of metal from roots to the shoots (Shen et al., 1997; Chen and Cutright, 2001); (2) enhanced availability of metals in the rhizosphere resulting from changes of pH, redox potential, and excretion of organic acids and/or chelates by roots (Hinsinger, 2001); and (3) foraging for metals by the roots, involving preferential allocation of root biomass into regions of metal enrichment (Schwartz et al., 1999). In particular, little is known about the relative contributions of plant synthesized compounds that might affect the efficiency of metal transfer from root to shoot and the processes that occur in the rhizosphere. Moreover, the molecular processes controlling the dynamics of contaminants in the rhizosphere is poorly understood and this may be a reason for the current

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difficulty in enhancing the rate of metal uptake by plants. Knight et al. (1997) suggested that root exudates may play a role in hyperaccumulation, but few studies have been conducted specifically to test this hypothesis and the exact role of exudates in hyperaccumulation is still to be established (Knight et al., 1997; Luo et al., 2000). Plants can exhibit specific mechanisms when they are exposed to high concentration of heavy metals resulting in changes of soil pH and DOC/OAs concentrations in the rhizosphere ultimately influencing the metal solubility and their uptake.

In this study, the influence of hyperaccumulator plants on soil pH and DOC/OAs concentrations and their subsequent effect on heavy metal solubility in the rhizosphere was investigated using a rhizobox. In particular, the study was focused on: (1) the effect of roots on rhizosphere chemistry (pH, DOC, OAs, heavy metal availability), and (2) the transfer of root exudates and soluble metals between the rhizosphere and the bulk soil.

1 Materials and methods

1.1 Soil properties

Soils were either collected from the vicinity of a Pb and Zn smelter operating in Port Pirie, SA, Australia (PP02 and PP03), or from a military shooting range in South Korea (Ko01 and Ko02). Soils PP02, PP03, and Ko01 are metal contaminated soils while Ko02 was used as a non-contaminated control soil. The bulk properties of the soils are summarised in Table 1.

Soil pH and EC were determined on a 1:5 of soil:water (W/V) suspension using a combination pH-EC meter (smartCHEM-LAB, TPS, Australia) following one hour equilibrium with shaking on an end-over-end shaker. Soil organic matter content was determined using the Walkely-Black method (Nelson and Sommers, 1996) and soil texture was determined using a micro-pipette method (Miller and Miller, 1987). Total heavy metal concentrations in soils were determined by ICP-MS following microwave (MARS5, CEM, USA) assisted aqua regia acid digestion in accordance with Method 3051H (USEPA, 1997). All digestions were conducted in triplicates and each batch included a standard reference material (Montana Soil SRM2711, Certificated by National Institute of Standards & Technology) and blank to validate the digestion procedure.

1.2 Rhizobox

A rhizobox allows for the separation of soil layers at a defined distance from the roots. In this work, a rect-

angular rhizobox was modified following Wang's design and constructed from five individual petri dishes glued end to end after replacing the petri dish bottom with nylon mesh (300 mesh) (Wang et al., 2002). The final constructed rhizobox (90 mm × 80 mm × 90 mm) consisted of 5 partitions separated by nylon mesh between each section and 10 small holes (1 mm diameter) were made on the base (under the frame) of the each section to supply water from saucers. The middle section was designated the rhizosphere zone and both side sections of rhizosphere zone were designated the near-rhizosphere while the outer sections were designated the bulk soil zone. The outside of the rhizobox was wrapped with black plastic film to prevent entry of light (Fig. 1).

1.3 Plant cultivation

The soil sample (200 g, < 2 mm) was loaded into each section of the rhizobox making the total weight of soil per rhizobox equal to 1 kg. A basal fertilizer solution (150 mg N/kg using NH_4NO_3 , 75 mg P/kg and 95 mg K/kg using KH_2PO_4) was supplied from the saucer through the holes in the base of the rhizobox until soils were saturated. After one week, water was added through the holes to replace the moisture loss due to evaporation and the soils allowed to equilibrate for 48 hrs.

Fifteen Indian mustard (*Brassica juncea*) seeds were sown in the rhizosphere zone of each rhizobox and the rhizoboxes were covered with transparent plastic bags to maintain the moisture content during germination. The plastic bags were removed 3 days after germination and the number of seedlings thinned to 8 per rhizobox. The seedlings were watered every 3 days from the bottom of the rhizobox through the holes for initial 2 weeks of growth and subsequently watered every 4 days until harvest. Watering was continued until the soils were saturated. Five weeks after germination, the plants were harvested. The experiments were conducted with four replicates for each soil type.

1.4 Soil solution extraction and analysis

After plant harvest, the soils in each section from each rhizobox were air dried separately and soil solution was extracted at 70% maximum water holding capacity (MWHC). The soil sample (20 g, < 2 mm) was weighed into 20 mL disposable plastic syringe plugged with acid washed glass wool. The syringe was inserted into 50 mL centrifuge tube separated from the base by a PVC spacer (Thibault and Sheppard, 1992). Water was added to the soil to obtain 70% of the MWHC and the soil was allowed to equilibrate for 24 hr. The resulting soil paste was

Table 1 Properties of bulk soils used in the present study

Soil	Soil pH	Organic matter (%)	DOC (mg/L)	Clay (%)	Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
PP02	9.2	0.3	16	12	17.8	446	2427	6472
PP03	8.2	1.7	613	13	3.6	630	625	9759
Ko01	6.2	0.6	15	18	7.9	87	380	55
Ko02	6.4	1.1	58	7	0.1	3.4	24	18

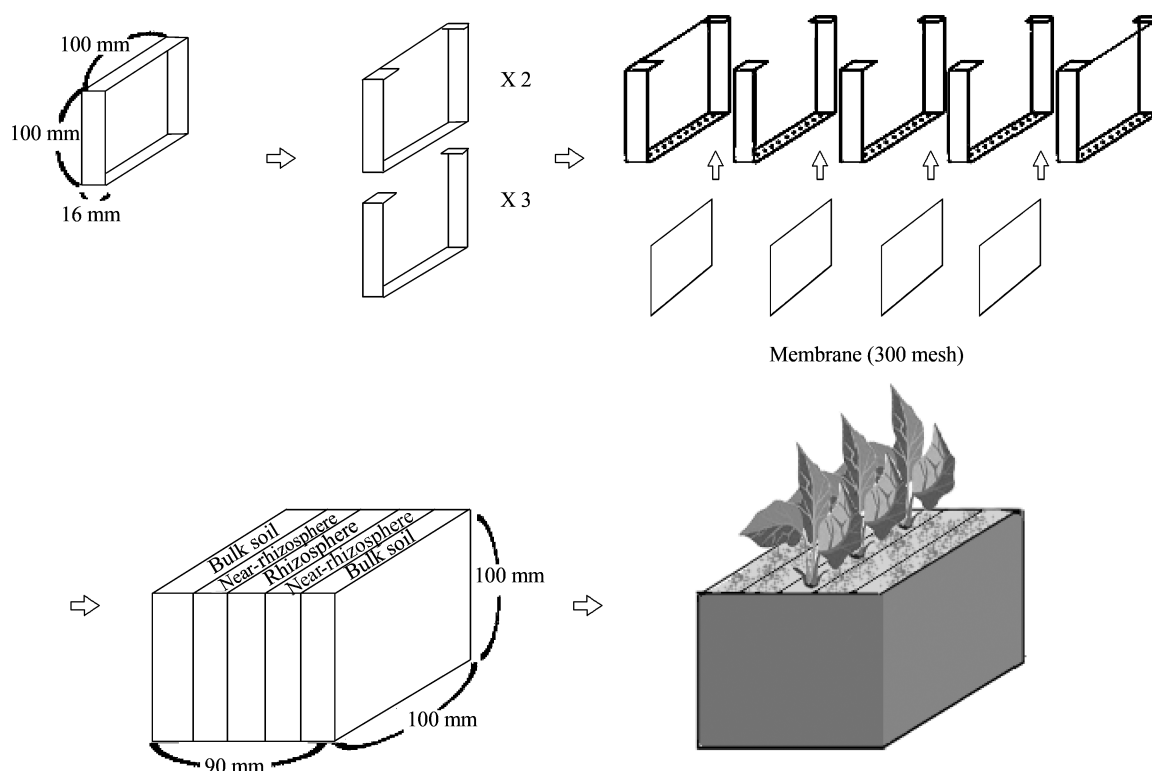


Fig. 1 Diagram of rhizobox design.

centrifuged at 2500 r/min for 25 min. and the isolated soil solutions were filtered through 0.45 μm cellulose acetate disposable filters (MillexTM, Millipore, USA).

The extracted soil solutions were used for the determination of pH, DOC, OAs and cation concentrations. Soil solution pH was determined using a combination pH-EC meter (smartCHEM-LAB, TPS, Australia). Total dissolved organic carbon (DOC) in soil solution was analysed using an automatic total organic carbon analyser (Model 1010, O.I. Analytical). Cations including heavy metals in extracted solution were analysed using ICP-MS and OAs were analysed using ion chromatography (ISC-2000, Dionex, USA) on an AS19 column (Dionex, USA).

1.5 Microbial counts

The population of bacteria were enumerated as colony forming units (CFU) from 10 fold serial dilutions of the soils (in duplicates) prepared in phosphate buffered saline plated out in duplicate agar plates and colonies counted after 5 days of incubation at 26°C. The bacterial population was estimated using quarter-strength Tryptic soy agar medium (Megharaj et al., 2000).

1.6 Data analysis

All data were statistically analysed using SPSS (12.0.1) to examine relationships between changes in metal concentrations in soil solution of the rhizosphere soil and key soil solution properties. For the regression analysis, individual data were used instead of average of four replications.

2 Results and discussion

2.1 Effect of plant roots on soil solution pH

The effect of plant roots on the rhizosphere soil pH varied considerably amongst the soils. The growth of Indian mustard resulted in an increase in pH, and the degree of increase depended largely on the magnitude of the original pH values and the distance from the bulk soil. Thus the two alkaline soils with pH exceeding 8 showed only a small increase in pH compared to the acidic soils in which the rhizosphere soil solution pH increased by 0.6 units compared to the bulk soil (Fig. 2). Changes in soil solution pH in the rhizosphere have been attributed to root exudates resulted from imbalance uptake of anion and cation by plants with the impact of this being reduced as the distance from the root increasing. The soil pH changes in the rhizosphere are related to the balance of cation or anion uptake and concomitant release of H^+/OH^- and organic acids (Sas et al., 2001). The increases in soil solution pH in the rhizosphere can impact the chemistry of heavy metals at the root:soil interface.

2.2 Effect of plant roots on DOC

Dissolved organic carbon in soil solutions is operationally defined as the fraction of organic carbon that can pass through a 0.45- μm membrane filter (Herbert and Bertsch, 1995). Irrespective of soil type there was a significant ($P < 0.01$) increase in DOC in the rhizosphere (Fig. 3).

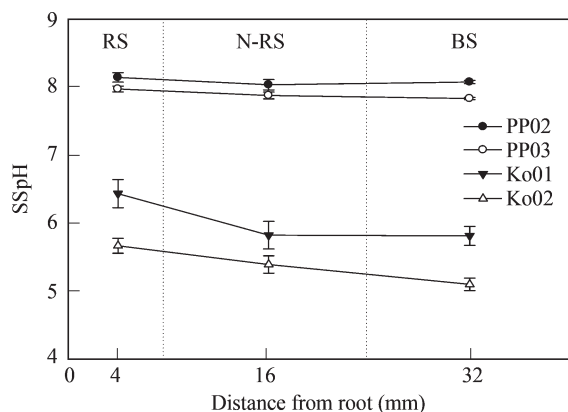


Fig. 2 Influence of plant roots on soil solution pH (SSpH) at a distance from root. RS: rhizosphere; N-RS: near-rhizosphere; BS: bulk soil.

The concentration of DOC in the rhizosphere can be affected by both plant root exudates and microbial activities. As shown in Fig. 3, the DOC in soil solution was more than double in the PP03 soil rhizosphere compared to other soils. A smaller effect of plant roots on rhizosphere DOC was recorded in other soils, and these changes in DOC appeared to have impacted on microbial activity (data not shown). Investigation of the microbial populations estimat-

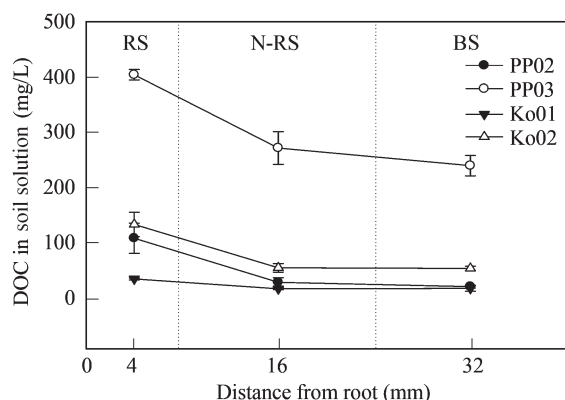


Fig. 3 Influence of plant roots on DOC concentration in soil solution at a distance from root.

ed as number of bacterial colony forming units showed a strongly positive relationship with the concentration of DOC in the rhizosphere after plant culture ($r^2 = 0.97$, $P < 0.001$). The bacterial populations were higher in rhizosphere soils than that in bulk soils. This suggested that the observed DOC increase in the rhizosphere could be due to both the plant root exudates and microbial populations. It is well known that the root exudates serve as carbon source to heterotrophic microbial populations. A highest number of bacterial populations were recorded in the rhizosphere soil of PP03 compared to other soils.

Increased DOC observed in the near-rhizosphere provided evidence that plants roots could also have impact on the soil solution chemistry of the near-rhizosphere soil especially when the changes are high enough to be detected as in soil PP03. However, the migration of DOC from the rhizosphere to bulk soil depends on other soil factors such as microbial activity and adsorption (Mench et al., 1988). Similar increase in DOC was recorded for the other soils although the magnitude of increase was much lower in Ko01 soil (Fig. 3). The increase in rhizosphere DOC would consequently influence metal solubility.

2.3 Effect of plant roots on soil solution organic acids

Among the various root exudates, organic acids (OAs) are of particular importance due to their metal chelation/complexing properties for mobilization of mineral nutrients and heavy metals such as Cd (Wenzel et al., 2002). Organic acids are negatively charged anions which have the capacity to complex with metal ions in both the aqueous and soil solid phases (Jones, 1998).

The total OAs contents are strongly dependent on the total soil organic matter and DOC (Hees et al., 2000), and this was also demonstrated here in PP03 soil which had the highest OAs concentration. In general, total OAs ranged from 1.3 mg/L in the Ko01 soil to 140 mg/L in the PP03 soil (Table 2). Regardless of the soil type, the highest total OAs was recorded in the rhizosphere and decreased markedly with increasing distance from plant root. Increased OAs contents in the rhizosphere were

Table 2 Influence of plant roots on organic acid concentrations in soil solution from different soil zones

Soil zone	Organic acids (OAs) in soil solution (mg/L)						Total-OAs
	Lactate	Pyruvate	Tartarate	Oxalate	Citrate	Trans-aconite	
PP02							
Rhizosphere	26 (8*)	ND	5 (1)	2.1 (0.6)	12 (6)	0.09 (0.04)	44 (10)
Near-rhizosphere	10 (1)	ND	5 (1)	ND	0.1 (0.1)	0.10 (0.04)	15 (2)
Bulk soil	9.0 (0.6)	ND	2 (2)	ND	0.1 (0.0)	0.06 (0.01)	11 (2)
PP03							
Rhizosphere	8 (5)	ND	N.D	5 (1)	120 (21)	ND	140 (20)
Near-rhizosphere	5 (1)	ND	N.D	4 (1)	94 (10)	ND	104 (7)
Bulk soil	4.5 (0.2)	ND	N.D	4 (1)	100 (20)	0.02 (0.03)	110 (20)
Ko01							
Rhizosphere	2.1 (0.3)	1 (1)	N.D	0.1 (0.1)	11 (2)	ND	14 (3)
Near-rhizosphere	1.2 (0.7)	0.8 (0.2)	N.D	0.3 (0.0)	3 (3)	ND	5(4)
Bulk soil	0.9 (0.6)	ND	N.D	0.4 (0.1)	0.1 (0.1)	0.02 (0.02)	1.3 (0.5)
Ko02							
Rhizosphere	4 (2)	2 (2)	0.3 (0.7)	0.9 (0.2)	31 (9)	ND	40 (10)
Near-rhizosphere	2.1 (0.4)	ND	N.D	0.5 (0.2)	5 (4)	0.01 (0.01)	8 (4)
Bulk soil	1.7 (0.3)	ND	N.D	0.5 (0.2)	0.1 (0.1)	0.01 (0.02)	2.3 (0.5)

ND: not detected; * the data in parenthesis are standard deviations.

attributed to the release of exudates by roots at the soil:root interface.

Determination of dissolved organic acids demonstrated the presence of lactate, pyruvate, tartarate, oxalate, citrate, and trans-aconite in all soils and in particular in the rhizosphere. Among these organic acids, lactate and citrate concentrations in the rhizosphere were significantly increased due to root exudation ($P < 0.05$ for lactate and $P < 0.001$ for citrate). However, the highest increase in OAs was observed in the high pH and highly contaminated soils of PP02 and PP03. This is further supported by our recent hydroponic study which showed elevated OAs exudation as the treated Pb concentration increased (Kim et al., 2009).

Numerous researchers have attributed the increased root exudate release in the presence of contaminants solely to an induced toxicity coping response by plants (Bartlett and Regio, 1972; Delhaize et al., 1993). The observation that there was also a significant ($P < 0.01$) increase in OAs concentrations in the rhizosphere of the uncontaminated control soil (Ko02), suggesting that there may be some other factors also influencing the release of root exudates. Tartarate was observed only in PP02 and the rhizosphere of Ko02 while pyruvate was detected in the rhizosphere of both Ko01 and Ko02 soils. This implied that the OAs exudation mechanism of Indian Mustard worked differently depending on either the soil type or the nature of microflora. The effect of soil type on the quantity of OAs in the rhizosphere soil was also noted in the study by Cieřliński et al. (1998).

2.4 Effect of plant roots on soil solution chemistry of heavy metals

Unlike most studies that focussed on single metal system in soils, the present study includes soils that have

been contaminated with multi metals such as either Cd, Cu, Pb and Zn (PP02 and PP03) or Cd and Pb (Ko01 and Ko02). Given the presence of multiple heavy metals the chemistry of these elements in the soil are influenced by both competitive adsorption arising from the widely different metal binding capacities and also from changes in the DOC and pH due to root exudates released by plant roots.

In general, under similar conditions, the relative affinities of metals tend to follow the Irving Williams series. Thus binding of heavy metals present in the soils used for present study will follow the order: $\text{Pb}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Cd}^{2+}$. This suggests that the presence of Pb will impact the chemistry of the other three metals. Thus biochemical changes in the rhizosphere that enhance binding of Pb will cause the release of Cu, Zn or Cd in soil solution.

2.4.1 Heavy metal dynamics in rhizosphere

Overall, Indian Mustard culture resulted in an increase of soluble heavy metal concentrations in the rhizosphere of the alkaline soils (PP02 and PP03) and decrease of soluble heavy metal concentrations in the rhizosphere of acidic soils (Ko01 and Ko02) with the exception of Cu, which also showed an increase in acidic soils (Fig. 4). These change are attributed to variation in the soil solution chemistry such as pH and DOC concentrations as affected by plant root exudation. Thus, the metal solubility in near-rhizosphere soil is also expected to be influenced by the rhizosphere.

In alkaline soils, PP02 and PP03, all heavy metal solubilities were increased in the rhizosphere after plant culture, even though there was some potential that soluble heavy metal could be depleted by plant uptake and adsorption onto soil surface induced by a slight pH increase. This could be due to measured significant increases in DOC in these soils that might be facilitating the release

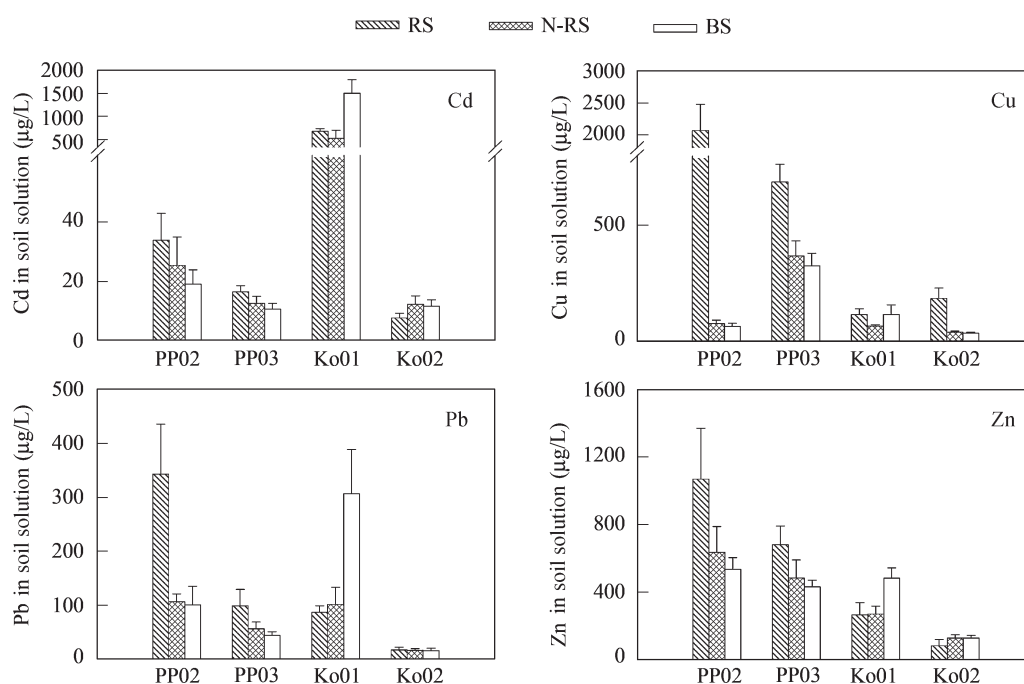


Fig. 4 Concentrations of heavy metals (Cd, Cu, Pb, and Zn) in soil solutions of the different soil zones.

of adsorbed heavy metals from the solid phase to the soil solution as metal-DOC complexes. The DOC effect on metal solubility in alkaline soils has been previously reported (Naidu and Harter, 1998; Weng et al., 2002; Schmidt, 2003).

Under multi metal contamination, compared to Cd and Zn, the solubility of Cu and Pb in alkaline soils showed a much greater increase in rhizosphere after plant culture (Fig. 4), which implied that selective complexes with DOC were formed due to the higher affinity of these metals for DOC. In addition, the metal solubility in the rhizosphere was increased more in PP02 than in PP03 despite a higher DOC concentration in PP03. One reason for this could be the competition between cations in the soil solution for the DOC binding sites. Dissolved organic carbon induced by roots in PP02 may react readily with heavy metals in this soil system because of less competition for DOC binding sites by other ions, while in PP03 a high portion of DOC exuded from the root reacted with different cations such as Mg and Ca. Total dissolved cations including Mg and Ca were 3 times greater in PP03 than that in PP02. In terms of solid-phase adsorption of heavy metals, the effect of index cations on sorption can be manifested through both direct competition for adsorption sites and its effect on the diffusion double-layer chemistry (Naidu et al., 1994; Harter and Naidu, 2001). In these studies, the adsorption of metal cations was decreased in the presence of Ca ions. This mechanism would also be applicable for metal complexation with dissolved ligands in solution phase which may increase metal solubility in alkaline soils as observed in PP02.

In contrast to alkaline soils, the Cd, Pb and Zn solubility in the rhizosphere of acidic soils (Ko01 and Ko02) was decreased under the influence of Indian mustard (Fig. 4). The observed decrease in metal solubilities in the rhizosphere of acidic soils may be attributed to the increase in soil solution pH regardless of increase in DOC, implying that the metal solubility in these acidic soils was mainly governed by soil pH. As shown in Fig. 2, soil solution pH in these soils was significantly increased by root operation. An increase in pH enhanced heavy metal binding onto the soil surface through deprotonation (Naidu and Harter, 1998). Nevertheless, the lower decrease in soluble metals in the rhizosphere of Ko02 is probably due to the greater increase in DOC in this soil compared to Ko01. The increased DOC may outweigh the influence of pH on metal solubility. Sauvé et al. (1998) reported the traditional increase in Pb solubility with decreasing pH below pH 6.5. However, above pH 6.5, they observed that the increased DOC in soil solution formed DOC-Pb complexes, resulting in increased Pb solubility as the pH increased. There was a concern that at near neutrality and above, the Pb in solution would be more mobile (Sauvé et al., 1998). These observations are consistent with the results obtained in the present study where Pb solubility decreased in acidic soils (Ko01 and Ko02), and increased in alkaline soils (PP02 and PP03).

Unlike other metal ions, soluble Cu increased in the rhizosphere of the acidic soil, Ko02, despite a significant

increase in the rhizosphere soil solution pH. Soluble Cu-organic complexes have been shown to control most of the solubility of Cu over a wider range of pH (McBride and Blasiak, 1979; Nederlof and Van Riemsdijk, 1995).

2.4.2 Metal ion migration

There were slight increases in soluble heavy metals in the near-rhizosphere soil of PP02 and PP03 compared to those in bulk soils (Fig. 4). This is probably due to the migration of DOC secreted from the root to the near-rhizosphere and subsequently increased dissolution of metal ions in the near-rhizosphere. This increase may also be partially due to increased DOC-metal complex formation in the rhizosphere and subsequent migration to the near-rhizosphere because of the gradient of soluble metal concentrations. This was supported by observed higher DOC concentration in the near-rhizosphere zone compared to bulk soil zone (Fig. 3).

In the near-rhizosphere of Ko01 there was no significant difference in soil solution pH and DOC from that of the bulk soil. Nevertheless, soluble Cd decreased significantly in the near-rhizosphere soil compared to bulk soil (Fig. 4). This may be due to the migration of soluble Cd from near-rhizosphere to rhizosphere as the amount of soluble Cd is decreased by pH increase and Cd uptake by Indian mustard in rhizosphere. Eventually, the decrease in soluble Cd induced by pH increase with Indian mustard culture resulted in continuous Cd influx to rhizosphere from surrounding soils as it evidenced from lower Cd concentration in near-rhizosphere than in bulk soil. The transport of metal ions from bulk soil to rhizosphere soil was observed previously when determining NH_4NO_3 extractable Zn in rhizosphere and bulk soils using a rhizobag system (Whiting et al., 2001). Onyatta and Huang (2003) suggested that removal of a metal ion by plant uptake establishes a concentration gradient to transport more metal ions toward the root surface. Similar observations were made for Pb and Zn with slight increase of the metal solubility in the near-rhizosphere of alkaline soils.

2.4.3 Multi metal effect

Heavy metals that have a higher affinity for binding to soil colloids could influence the solubility of the lower affinity metals through direct competition for available binding sites. Kim et al. (2009) reported that the presence of Cu and Pb directly affected Cd and Zn solubility. Higher concentrations of soil solution Cu and Pb also resulted in higher Cd and Zn concentrations in soil solution of long-term contaminated soils. Likewise, the changes in heavy metal solubility due to the culture of Indian mustard when simultaneously exposed to multiple heavy metals were influenced not only by changes in physiochemical rhizosphere properties, such as pH and DOC, but also by magnitude of the change in soil solution concentration of higher affinity heavy metals such as Pb and Cu.

As shown in Fig. 5, the change in rhizosphere Zn concentration showed a positive relationship ($r^2 = 0.87$, $P < 0.001$) with the change in soil solution Pb indicating that increased Pb concentrations resulted in the release

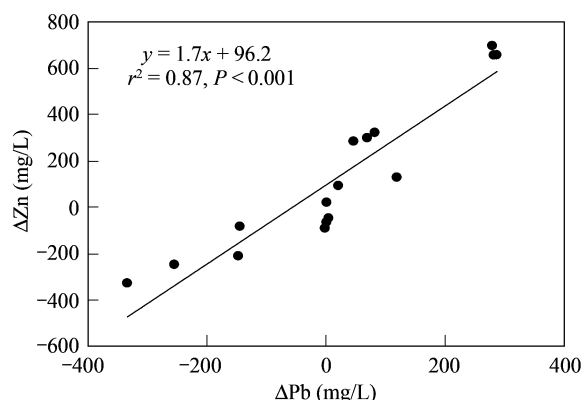


Fig. 5 Relationship between the change of Pb (ΔPb) and Zn (ΔZn) in the rhizosphere after Indian mustard culture. Δmetal = rhizosphere metal concentration – bulk soil metal concentration.

of more Zn from the solid phase into liquid phase. The coefficient of variation for this relationship was improved by the change in pH ($r^2 = 0.91$, $P < 0.001$) as already demonstrated in our previous study (Kim et al., 2009). This indicates that the effect of Pb on Zn solubility was also related to the pH change. Inclusion of the change in DOC concentrations did not improve the relationship suggesting that DOC was not as important as either pH or Pb in determining Zn solubility.

The increase in Pb concentration in soil solution after Indian mustard culture also influenced the solubility of Cd. Increases in Cd concentration were positively correlated ($r^2 = 0.68$, $P < 0.001$) with Pb and the correlation was slightly improved by either pH ($r^2 = 0.72$, $P < 0.001$) or DOC ($r^2 = 0.76$, $P < 0.001$) as regression parameters.

Consistent with Irving Williams series changes in Zn concentration following Indian mustard culture significantly correlated with the changes in Cu concentration ($r^2 = 0.72$, $P < 0.001$). Additional Zn was released from the solid phase as Cu concentration increased in the rhizosphere soil solution ($r^2 = 0.78$, $P < 0.001$). As observed for Pb, as a fitting parameter pH also improved the coefficient of variation for the Cu-Zn relationship ($r^2 = 0.82$, $P < 0.001$).

3 Conclusions

When Indian mustard was cultured in a rhizobox, significant changes in both pH and DOC were observed. These chemical changes were identified as being principally responsible for subsequent changes in metal solubility. After Indian mustard culture, a higher pH increase was observed for acidic soils and a higher DOC increase was observed in alkaline soils. In the alkaline soils, DOC was the main factor governing metal solubility with the observation of increased metal solubility as DOC increased following Indian mustard culture. In contrast, metal solubility was decreased in acidic soils and this was attributed to the significant pH increase after plant culture despite concurrent DOC increases in these soils. The magnitude of metal solubility changes also varied with metal type. For instance, Cu solubility was more closely related with DOC than any other metals in the soil. In addition, due to

the co-existence of multiple metal contaminants in the soil system, an increase in Cu and Pb concentration following Indian mustard culture resulted in a release of Cd and Zn from the solid phase due to the competition for available binding sites between metals. Competitive binding was considered to favour Cu and Pb which have higher stability constants than Cd and Zn.

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