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Lead Induced Organic Acid Exudation and Citrate Enhanced Pb Uptake in Hydroponic System

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ABSTRACT: The influence of Pb-citrate complex formation on Pb uptake and the effect of Pb on organic acid exudation were investigated using four plant species, *viz.*, sunflower (*Helianthus annuus* L), Indian mustard (*Brassica juncea*), canola (*Brassica napus*) and vetiver grass (*Vetiveria zizanioides*) under hydroponic conditions. Seedlings were exposed to different levels of Pb and Pb-citrate for 24 hrs and subsequently Pb distributions in plant shoot, root and hydroponic solution were measured. The dissolved organic carbon (DOC) concentration generally decreased as the concentration of Pb in the hydroponic solution increased. In contrast to DOC, the total organic acid concentrations exuded from Indian mustard roots significantly increased (424 to 6656 mg kg^{-1}) with increased Pb treatment, implying that exuding organic acids were involved in Pb accumulation in Indian mustard. The complexation of Pb with citrate enhanced Pb accumulation in the above ground portions. Lead concentration in Indian mustard increased from 2.05 mg kg^{-1} to 6.42 mg kg^{-1} when the concentration of citrate in solution increased from 0 to 50 mg L^{-1}. This result showed enhanced translocation of Pb from root to shoot with observation of transfer coefficient (K_t) increase from 2.03E-3 to 5.72E-3.

Key Words: DOC, Indian mustard, organic acids, Pb, sunflower, canola, transfer coefficient (K_t)

Introduction

It is well known that plant roots may exude organic carbon in the form of a wide variety of organic compounds which are synthesized during photosynthesis and translocated into the roots[1,2)]. Plant roots may also exude counter ions such as H^+ and OH^- for compensating charge balance in the inner sphere of cells when they take up elemental nutrients, which results in changes of soil solution pH[3)]. These root exudates may significantly influence on the properties of the soil environment in the vicinity of the rhizosphere[4-6)] including microbial activity, rhizosphere physical properties, pH and metal speciation[6-8)].

Numerous researches were conducted to understand the role of root exudates in metal translocation and to identify the specific compounds comprising root exudates[4,9-11)]. From these studies low molecular weight (LMW) compounds such as amino acids, organic acids, sugars, phenolics, and various other secondary metabolites are believed to comprise the majority of root exudates, with high molecular weight (HMW) compounds including mucilage and proteins being of secondary importance[9,11,12)]. The exact constitution of any specific root exudate is variable being highly dependent upon plant species and soil type.

The investigation of root exudates can be approached in two different ways 1) alleviation of heavy metal toxicity or 2) increase in heavy metal accessibility to plants[13-16)]. It is generally accepted that free metal ions are more toxic and more accessible to

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plants than organically bound metals. Hence, plant roots may exude organic compounds as a protection mechanism to transform toxic heavy metals to organo-metal complexes and thus alleviate toxicity[14,16,17].

The transformation and increase in heavy metal solubility due to root exudates are understood to be one of the most plausible mechanisms responsible for hyperaccumulation of heavy metals by some plants[18-20]. Low molecular weight organic acids (LMWOAs) among the compounds comprising root exudates have relatively high affinity for heavy metals resulting in desorption of heavy metals from soil surfaces into soil solutions followed by increase in heavy metal uptakes by plants[7,21-23]. Moreover, organic acids in plant tissue enhance the transport of heavy metals from root to shoot[14,24]. For instance, levels of citric, malic, malonic and oxalic acids in hyperaccumulators have been correlated with elevated concentrations of Ni or Zn in the tissue[25]. Even though there have been many root exudate studies, the role of root exudates on metal dynamics in the rhizosphere has often been overlooked and consequently still not well understood. For this reason the identification of root exudates and their role from some candidate plants that may be suitable for applications of practical phytoremediation is necessary.

In addition, investigation of the main metal species taken up by plant roots would be necessary to understand the root mechanism of hyperaccumulator plants. This is because controversy still exists as to whether plants uptake free metal ions only or metal ions complexed with LMWOAs as well as free ions, despite the free metal ion fraction in soil solution still being the most important for plant uptake[18,26,27].

The objectives of the present study were 1) to identify and quantify organic acids exuded from roots of different plant types in response to different Pb levels, 2) to determine the influence of those organic acids on metal transformation and plant uptake, and 3) to investigate the main heavy metal forms transferred through the plant roots.

Materials and Methods

Plants

Indian mustard (*Brassica juncea*) is a known hyperaccumulator for Zn and Cd have the potential for enhanced Pb accumulation, while canola (*Brassica napus*) which is from the same family (*Brassicaceae*) does not hyperaccumulate these metals. These two plants were selected to investigate the different response of a hyperaccumulator and a non-hyperaccumulator to elevated Pb concentration in hydroponic solution. Sunflower (*Helianthus annuus* L.) and vetiver grass (*Vetiveria zizanioides*) were also selected for this study because they have the potential to be used for the stabilization of heavy metals around the root zone and may have very different mechanisms for interacting with metals than the hyper- and non hyperaccumulating plant species.

Experiment 1

In this experiment a hydroponic system was used as a pure system, relative to that of the more complicated soil matrixes, in order to identify and quantify organic acid and dissolved organic carbon (DOC) concentrations potentially exuded from plant roots in response to exposure to heavy metals.

Canola, Indian mustard and sunflower were propagated from seeds. Seeds were germinated on filter paper in a petri-dish and subsequently transplanted into hydroponic system containing 1/5 strength Hoagland's solution which was aerated for 20 min every hour. Vetiver grass was propagated from sprouts obtained from Veticon Consulting Pty. Ltd. (Queensland). All seedlings except vetiver grass were grown for 3 weeks under a photoperiod of 16 hr, light intensity of 850 lux, and 26℃ prior to 24 hr exposure measurements being conducted. Vetiver grass was grown for 4 weeks before treatment.

For the 24 hr exposure treatments, 3 week or 4 week old seedlings were transferred into individual treatment containers (100 mL) having five different levels of Pb solutions (0, 0.5, 1, 2, and 5 mg L^{-1}). This treatment was conducted with and without 1/5 strength Hoagland's solution. Deionized water was used to make the nutrient solution. All experiments were performed in triplicate. One replicate constituted 1 seedling to 1 exposure treatment container (100 mL).

After 24 hr exposure, plants were harvested and separated shoots (above ground) and roots, rinsed with Milli-Q water several times and dried at 65℃ in a fan forced oven. The dried shoots and roots were weighed to allow calculation of concentration based on dry weight. Nutrient solutions were collected and filtered through 0.45 μm cellulose acetate disposable filter (Millex™, Millipore) prior to analysis for Pb, DOC, and organic acid content. The volume of treatment solution remaining after harvest was also measured so that the amount of organic acid and DOC could be calculated and they were compared to the concentration based on root dry weight.

Experiment 2

In order to investigate the species of Pb (either free Pb or Pb complexed with organic compounds) transferred through the plant roots, the fraction of free and complexed Pb in 1/5 strength of Hoagland's solution containing a total Pb concentration of 500 μg L^{-1} were adjusted by adding known amounts of tri-sodium citrate. Citrate is a ubiquitous LMW chelate that forms high stability complexes with Pb. For this experiment, the seedlings were prepared and transplanted in each treatment container (100 mL) with the same way for Experiment 1 as described above. The experiment was conducted in triplicate. Plants were harvested after 24 hr treatment and analyzed for Pb concentration in plant tissue.

Treatment levels

The treatment levels of lead were established through simulation using MINTEQA2 to control the Pb species and to prevent supplied Pb (as $Pb(NO_3)_2$) from precipitation with phosphate. In addition, the Pb concentration of the soil solution (200 to 500 μg L^{-1}) extracted from Pb contaminated soils which will be used for further study was considered to determine the Pb treatment concentration. The different citrate input levels for experiment 2 were established after simulating the Pb species fractions together with citrate concentration using MINTEQA2 and also Pb^{2+} was measured practically using donnan membrane technique (DMT) (Table 1). In order to control the Pb species in solution, EDTA, one of the components for Hoagland's solution was excluded in preparation of the solution because it formed highly stable complexes with supplied Pb resulting in difficulties in altering Pb species.

Analysis

Dried and ground plant tissue (0.5 g) was digested with concentrated HNO_3 (5 mL) using a digestion block (AIM 500, A. I. Scientific). The digested solution was diluted 20 times with Milli-Q water before filtering with disposable cellulose acetate 0.45 μm filters. The filtered solutions were stored in cold room at 4℃ until analyzed for total Pb concentrations using ICP-MS (Agilent, 7500 series). Organic acids in nutrient solutions were quantified with ion chromatography (IC, Dionex, ISC-2000) fitted with AS19 column (Dionex) after filtering through 0.45μm disposable cellulose acetate syringe filters. The type of detected organic acids was confirmed using HPLC (Agilent, 1050) with the method developed by Chen et al.[28]. DOC was determined using a total organic carbon analyser (Model 1010, O.I. Analytical). Free Pb concentrations in solution were determined using a modified DMT technique[29]. The results were compared with the calculated Pb species simulated using MINTEQA2. Total Pb concentrations in nutrient solution and plant tissue digestion solution were determined using ICP-MS with a Ru internal standard

Table 1. Concentration of treated citrate and fraction of corresponding Pb^{2+} and Pb-citrate compound determined by DMT and MIMTEQA2 in solution

Citrate	Pb^{2+} by DMT[a]	Pb^{2+} by MTQ[b]	Pb-citrate by MTQ[b]
(mg L^{-1})	(%)		
0	72.5	75.8	
1	48.7	64.4	14.9
5	27.4	55.4	26.8
10	7.1	42.1	44.3
50	0.6	20.9	72.1

a. Pb^{2+} determined by donnan membrane technique (DMT)
b. Pb^{2+} simulated by MINTEQA2

and periodic determination of quality control standards and blanks to ensure accuracy of determination.

Calculations

The total concentration of Pb in plant tissue, organic acid, and DOC exuded from plant root to nutrient solution were calculated on a dry weight basis. The transfer coefficient (K_t) which quantifies transfer of Pb from root to shoot was determined using:

$$K_t = \frac{M_S}{M_R}$$

Where, M_R and M_S were the dry weight concentrations of metal in root and shoot, respectively in mg kg^{-1}.

Results and discussion

Lead uptake

As with previous studies[30,31] Indian mustard accumulated the highest Pb concentration in their shoot and root among the plant species studied in the present study (Fig. 1). However, the shoot concentrations recorded in this study were significantly less than those reported in previous studies[30,31].

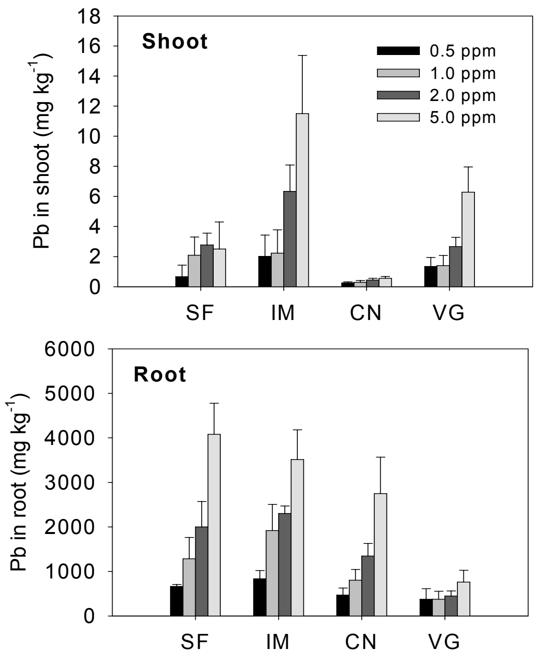

Fig. 1. Lead concentration in shoot and root after treatment in different Pb concentrations in solution for 24 hours. (sunflower, SF; Indian mustard, IM; canola, CN; vetiver grass, VG)

This may be attributed to the short exposure period. The concentration of Pb in Indian mustard shoot increased from 2.0 to 11.5 mg kg^{-1} DW while Pb concentration in root increased from 835 to 3513 mg kg^{-1} DW, with increasing Pb concentration in the treatment solution. In contrast, canola, a non-hyperaccumulator accumulated the lowest concentration of Pb in the shoot among all the plants studied ranging from 0.24 to 0.55 mg kg^{-1}.

A key feature of hyperaccumulators is higher translocation of metals from root to shoot (higher transfer coefficient, K_t). The K_t for Pb in Indian mustard was much higher than that in canola indicating higher translocation of Pb from root to shoot and consequential higher accumulation of Pb in above ground tissue (Fig. 2).

The K_t for Pb in Indian mustard increased when the plant was exposed to more than 2.0 mg L^{-1} of Pb in the treatment solution indicating that Indian mustard was responding to the change in Pb concentration outside of root and exhibiting a mechanism for uptake and translocation of Pb. In contrast, K_t for canola was the lowest among the plant species and showed a tendency of decrease with increase in Pb concentration in the treatment solution suggesting this species have no mechanism available to enhance Pb transfer to shoots.

Sunflower has a well developed root structure and has previously been applied for phytofiltration because of its high ability for metal adsorption on this extensive root surface[32]. In this study, sunflower accumulated elevated level of Pb in the root (663 to

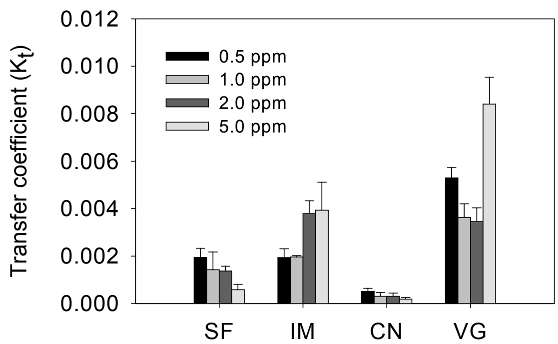

Fig. 2. Transfer coefficient (K_t) of plants (sunflower, SF; Indian mustard, IM; canola, CN; vetiver grass, VG) exposed in different Pb concentration solutions.

4077 mg kg^{-1} DW) when the Pb concentration in the treatment solution increased. However, the concentration of Pb in the shoot was less sensitive to changes of Pb concentration in solution, resulting in an overall decrease of K_t with Pb concentration (Fig. 2). Even the concentration of Pb in the shoot grown in the highest Pb treatment solution (5.0 mg L^{-1}) was lower than that from the 2.0 mg L^{-1} treatment presumably caused by physiological damage because of Pb toxicity.

Vetiver grass showed the lowest Pb concentration in root and the highest K_t. The highest K_t does not necessarily mean this species is good for phytoextraction. Vetiver grass has different root morphology and architecture from the other plants studied, having far less surface area and less lateral root likely causing less capacity for metal adsorption resulting in higher K_t.

DOC from root

The major source of dissolved organic carbon (DOC) in soil solution is exudates from plant roots[19]. Plant roots release considerable amounts of organic carbon into the soil as diverse compounds, including water soluble exudates such as sugars, organic acids, and amino acids together with water insoluble materials such as cell walls and mucilage[1]. These compounds can influence both the availability of elements in the soil solution and the uptake of elements by plants either directly by complex formation or indirectly through stimulating microbial activity[33,34]. This mechanism of organic compound exudation and consequential mobilization of metals is believed to be involved in hyperaccumulation by several studies[18-20].

Typically plants release approximately 25% of their assimilated carbon as root products into the rhizosphere[34], whereas the total concentration of DOC exuded from roots depends both on the plant species and the local soil environment. For instance, Prikryl and Vancura[35] and Barber and Lynch[36] reported the total amount of organic compounds released from roots was in the range of 0.5% and 20% of the total plant dry weight, respectively.

Among the plants studied, sunflower exuded the highest amount of DOC with a range of 9.0 to 13.5 g kg^{-1}, while the lowest DOC was observed in vetiver grass with a range of 0.31 to 0.61 g kg^{-1} (Fig. 3).

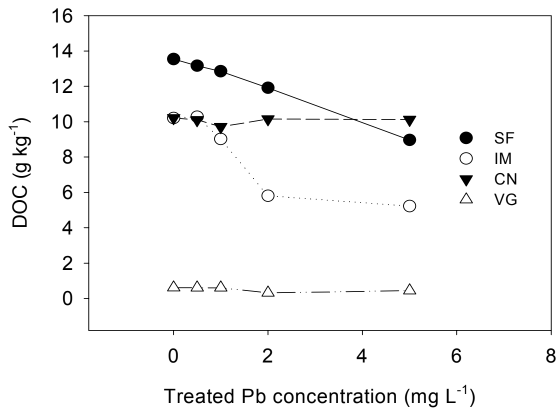

Fig. 3. Final dissolved organic carbon (DOC) concentrations in solution (g kg^{-1}) after plant harvest (sunflower, SF; Indian mustard, IM; canola, CN; vetiver grass, VG).

The DOC concentration exuded from Indian mustard declined from 10.2 to 5.2 g kg^{-1} with increasing Pb concentration in treatment solution, with the biggest drop observed when the treatment Pb concentration increased from 1.0 to 2.0 mg kg^{-1}, despite increased Pb uptake as treatment Pb concentration increased. This implied that the quantity of released organic compounds alone was not responsible for accumulation of Pb in Indian mustard. In addition, the DOC exuded from canola, the non-hyperaccumulator, remained constant around 10 g kg^{-1} with increasing treatment of Pb concentration implying that there was no additional exudation under the circumstance of external elevated Pb to alleviate Pb toxicity.

However, results from hydroponics could be significantly different from those of pot experiment using contaminated soils because metals are bioavailable and can readily access the root surface through diffusion and mass flow in hydroponic systems. The difference can also be resulted from the fact that no disturbing compounds such as negatively charged mineral particles and organic compounds exist in hydroponic system.

Organic acids from root

The total carbon present in solution in the form of organic acids comprised between 0.6-1.3, 0.6-50.3, 5.5-9.9, and 3.7-8.0% of the total DOC in sunflower, Indian mustard, canola and vetiver grass, respectively (Table 2).

These values were similar to those reported in

Table 2. Fraction (%) of total carbon in organic acids to DOC in solution after plant growing

Treated Pb ($mg\ L^{-1}$)	Sunflower	Indian mustard	Canola	Vetiver grass
	---------------------- (%) ----------------------			
0	0.6	0.6	5.5	4.3
0.5	0.6	1.3	9.9	5.6
1.0	0.7	3.7	8.8	4.5
2.0	0.8	24.7	7.6	8.0
5.0	1.3	50.3	6.9	3.7

Table 3. Variation of total organic acid concentrations exuded from each plant with Pb treatment concentration ($mg\ L^{-1}$)

Pb Treatment ($mg\ L^{-1}$)	Sunflower	Indian mustard	Canola	Vetiver grass
	---------------------- ($mg\ L^{-1}$) ----------------------			
0	293.3	424.3	1510.0	239.1
0.5	281.1	514.1	2536.1	281.6
1.0	310.1	1015.4	2152.9	237.4
2.0	292.8	3709.5	1968.4	175.5
5.0	345.6	6656.4	1769.8	172.1

other studies which showed carbon as LMW carboxylic acids comprising less than 10%[37] and 6%[38,39] of total DOC in soil solution. Whereas Mench et al.[40] reported LMW compounds represented 58% of the total carbon of the soluble root exudates in a hydroponic study using maize. The present study showed a much lower fraction (or percent) of carbon in organic acids to total DOC than the result by Mench et al[41] with the exception of Indian mustard treated with higher Pb concentrations. For these treatments 24.7 and 50.3% of the total DOC consisted of carbons in organic acid. The generally higher percentages observed by Mench et al.[40] may be related directly to the longer treatment period used in their study and to different plant species studied.

The relative fraction of carbon present as organic acids to total DOC increased significantly (0.6 to 50.3%) in Indian mustard as the concentration of Pb in the treatment solution increased suggesting exuding organic acids presumably associate with the elevated Pb uptake in Indian mustard even though total DOC had decreased with Pb treatment (Fig. 3).

In the absence of Pb, the total organic acids exuded from the plants of each control decreased in the order: canola (1510 mg kg^{-1} DW) > Indian mustard (424 mg kg^{-1} DW) > sunflower (293 mg kg^{-1} DW) > vetiver grass (239 mg kg^{-1} DW) (Table 3). Although Strobel[11] suggested that the concentration of aliphatic LMW di-/tri-carboxylic acids in solution was positively correlated with DOC, there was no relationship between total organic acids and DOC in the present study (Table 3; Fig. 3). The total organic acids exuded from Indian mustard to nutrient solution increased dramatically despite decrease in a total DOC with increasing Pb concentration. This indicated that high accumulation of Pb in Indian mustard was related to exudation of more specific organic acids rather than the total amount of DOC exuded from the root.

So far, most of the studies on the role of organic acids on solubility and bioavailability of heavy metals have been conducted using organic acid extraction methods or soils artificially spiked with organic acids[6,7,22,42]. From these studies, it has been found that application of organic acids reduce the adsorption of metals onto the soil, resulting in increased metal availability[6,21]. Likewise, organic acids in root exudates can be highly influential on metal speciation in the soil and soil solution by altering chemical processes in soil through complexation reactions with metals in solution and ligand-exchange reactions at soil surface [13,41,43].

It is strongly believed that root exudation enhancing metal mobilization is involved in hyperaccumulation[18]. Cieśliński et al.[9] found that the high Cd accumulating cultivar of durum wheat, Kyle, had significantly higher amounts of LMWOAs in the rhizosphere soil than that of the low accumulating cultivar, Arcola, regardless of soil type, implying LMWOAs were responsible for greater Cd availability in the rhizosphere and subsequent accumulation of Cd in the Kyle cultivar[9]. Moreover, there were highly positive relationships between Cd concentration in shoot and LMWOAs in the rhizosphere in both Kyle (r^2=0.99) and Arcola (r^2=0.98). This implies that exudation of LMWOAs from the root enhanced Cd uptake by wheat. However, in hydroponic system similar to the present study, where most Pb exists in bioavailable form, organic acids exuded from root can directly reduce the free metal ion activity, which is significant because it is generally believed that the free metal ion is the more accessible form for plant uptake[18,26,27]. Thus increased exudation can possibly result in reduced Pb uptake by Indian mustard. However, in the present study, there was no significant influence on Pb uptake attributable to reduction of free Pb in solution due to exudation of higher organic acids in response to exposure of plants to higher Pb concentrations. This indicated that there was potentially another significant mechanism associated with exudation of organic acids, besides translocation of Pb in Indian mustard and this was to enhance Pb uptake using organic acids as Pb transporters. Rhizosphere organic acids can chelate free Pb in solution and subsequently the chelated Pb might be able to pass through the cell membrane at a higher rate because of the higher metabolic activity interface of root and organic acids on the root surface[44].

Although a large number of organic acids may be exuded by roots only a limited number of them would be associated specifically with Pb due to metal specific affinity of some ligands for some metals and complexation between metals for available ligands. For this reason, identification of specific organic acids capable of forming strong complexes with free Pb in nutrient solution containing plants is very important.

Nature of organic acids in the root exudates

Of the 11 organic acids investigated here, as being commonly found in plant exudates, only 6 (lactate, acetate, pyruvate, succinate, fumarate, and trans-acconite) were detected in the four plant species with three common organic acids (lactate, acetate, and pyruvate) being detected in all four plant species (Table 4).

Some organic acids were specific for different plants. Succinate was observed in sunflower and Indian mustard, while trans-acconite was detected in both Indian mustard and canola, and fumarate was detected only in canola. The concentration of organic acids was correlated with Pb concentration in solution. For sunflower and Indian mustard, lactate increased with increasing Pb concentration while this decreased with increasing Pb treatment for canola.

The major organic acid detected in Indian mustard was lactate but only when the Pb concentration in the hydroponic treatment solution was over 1.0 mg L^{-1}, after which lactate concentrations significantly increased (0 to 6000 mg kg^{-1}) with increasing concentration of Pb in the solution (Table 4). In addition to this, succinate and trans-acconite from Indian mustard were only induced with Pb treatment. This implies that exudation of lactate is associated with accumulation of Pb and that new compounds exuded from the root as exposed to Pb may also be involved in the uptake mechanism. In contrast to this, pyruvate decreased (270 to 100 mg kg^{-1}) with increasing Pb concentration in solution. Indeed pyruvate decrease with increasing Pb treatment concentration was common to all plants studied. Fumarate was the dominant organic acid present in canola and the concentration of fumarate increased significantly when canola was exposed to Pb in treatment solution except at the highest Pb concentration. The trans-acconite concentrations also generally increased with increasing concentration of Pb in treatment solution rising to a maximum of 700 mg kg^{-1} DW. In contrast to fumarate and trans-acconite, there was generally a decrease in lactate, acetate, and pyruvate from 100 to 60, 110 to 70, and 150 to 80 mg kg^{-1}, respectively as Pb solution concentrations in the treatments increased. It, therefore, seems that Indian mustard and the canola, both from the same family, have different responses to elevated Pb concentrations in solution and organic acid exudation may be one of the main mechanisms contributing to accumulation of Pb.

As with Indian mustard, lactate exuded from sunflower increased from 58 to 173 mg kg^{-1} as the Pb concentration in the treatment solution increased but by a significantly lower amount than that from Indian mustard was observed. Acetate and pyruvate decreased from 90 to 40 mg kg^{-1}, and 79 to 51 mg kg^{-1}, respectively. While the amount of organic acids exuded from sunflower was much less than those observed for Indian mustard, the type of organic acids exuded were significantly influenced by Pb treatment.

For vetiver grass, only lactate, acetate, and pyruvate, which were detected in all plants, were detected in much lower concentrations than any other plants. The concentration ranges for lactate, acetate, and pyruvate were 13-33, 24-45, and 116-204 mg kg^{-1}, respectively and there was no significant evidence for changes in

Table 4. Organic acid concentration in hydroponic solution after plant cultivation

Treated Pb ($mg\ L^{-1}$)	Sunflower	Indian mustard	Canola	Vetiver grass
	-----------------------	($mg\ kg^{-1}$)	----------------------	
	Lactate			
0	60 (10[a])	n.d	100 (70)	20 (10)
0.5	70 (10)	n.d	90 (30)	30 (10)
1.0	90 (40)	750 (50)	60 (40)	20 (10)
2.0	120 (70)	3000 (2000)	30 (10)	30 (10)
5.0	170 (40)	6000 (3000)	60 (40)	13 (9)
	Acetate			
0	90 (10)	160 (40)	110 (60)	36 (4)
0.5	70 (20)	130 (30)	100 (70)	45 (9)
1.0	80 (30)	70 (10)	60 (30)	40 (10)
2.0	60 (10)	90 (50)	50 (10)	30 (10)
5.0	40 (10)	440 (70)	70 (20)	24 (6)
	Pyruvate			
0	79.2 (0.6)	270 (70)	150 (40)	180 (30)
0.5	72 (5)	180 (70)	130 (60)	204 (3)
1.0	73 (6)	190 (90)	100 (60)	180 (50)
2.0	60 (20)	140 (90)	100 (50)	120 (30)
5.0	50 (20)	100 (30)	80 (40)	135 (5)
	Succinate			
0	70 (30)	n.d	n.d	n.d
0.5	60 (20)	20 (30)	n.d	n.d
1.0	70 (20)	10 (10)	n.d	n.d
2.0	50 (20)	10 (10)	n.d	n.d
5.0	80 (40)	n.d	n.d	n.d
	Fumarate			
0	n.d	n.d	900 (200)	n.d
0.5	n.d	n.d	1700 (300)	n.d
1.0	n.d	n.d	1900 (300)	n.d
2.0	n.d	n.d	1200 (700)	n.d
5.0	n.d	n.d	900 (200)	n.d
	Trans-acconite			
0	n.d	n.d	300 (400)	n.d
0.5	n.d	180 (40)	500 (600)	n.d
1.0	n.d	n.d	13 (9)	n.d
2.0	n.d	90 (70)	600 (300)	n.d
5.0	n.d	100 (70)	700 (500)	n.d

n.d = not detected
a. Standard deviation of four replicates

organic acid concentrations in response to exposure to higher concentrations of Pb.

In a previous study using a distilled water extraction method to isolate soil solution from the rhizosphere soil, the organic acids isolated from quack grass included acetic, formic, citric, malic, aconitic and oxalic acids with acetic and formic acids accounting for more than 75% of the total aliphatic acids identified[4]. In another hydroponics study using maize (*Zea mays* L.), succinic (40%), citric (30%), lactic (15%), and malic (15%) acids at the relative amounts indicated in parenthesis were detected[40]. Cieśliński et al.[12] examined the composition of organic acids from 5 different durum wheat cultivars in standard nutrient solution and reported that oxalic, fumaric, succinic, acetic and tartaric acids were found in root exudates with oxalic and acetic acids being the predominant acids, but when they determined the organic acids exuded from the same cultivar in Cd contaminated soils the predominant organic acids was different from those previously detected in the hydroponic study. For the soil experiments acetic and succinic acids accounted for 93.2, 88.4 and 84.2% of the total amount of LMWOAs present for the three soils studied[9]. This implies that the different mechanism for organic acid exudation by this plant was exhibited by the Cd exposure in soil.

Pb uptake associated with organo-Pb

Extremely efficient translocation of metals from roots to shoots is one of the key features that distinguish metal hyperaccumulators from non-hyper-accumulators. High accumulation of metals in plants via long-distance translocation of metal ions from root to shoots may be limited by binding of metal cations to exchange sites located on the xylem cell walls[45]. However, hyperaccumulators may overcome this limitation by formation of negatively or neutrally charged organo-metallic complexes. The possible formation of metal chelates or complexes in soils may result in effective transport of metal-organic complexes in plants[44]. Kramer et al.[24] found that histidine in xylem sap enhanced Ni translocation through formation of a Ni-histidine complex. Nigam et al.[5] found that Cd added with organic acids resulted in statistically significant increases in Cd accumulation in root and above ground parts of the plant in both sand and soil culture with relatively higher increases in the plants grown in sand. The formation of metal-organic acid complexes could be an important mechanism to increase metal uptake by plants even though free metal ions are more accessible to plants.

The concentrations of Pb in the above ground tissue of sunflower, Indian mustard and canola were all significantly increased as more citrate was added to the solution (Fig. 4).

The concentration of Pb in Indian mustard increased around 3 times from 2.05 mg kg^{-1} without citrate treatment to 6.42 mg kg^{-1} with 20 mg L^{-1} citrate treatment. Likewise, a five fold increase in Pb above ground tissue concentration (2.35 mg kg^{-1} to 10.28 mg kg^{-1}) was observed in canola when the citrate concentration in solution increased from 0 to 50 mg L^{-1}. In addition the Pb concentrations in roots of canola and Indian mustard were generally elevated with increased citrate concentration. This implies that the formation and uptake of Pb-citrate complexes may be more important than the concent-ration of free metal ions for metal accumulation. The increase in Pb plant tissue concentrations induced by

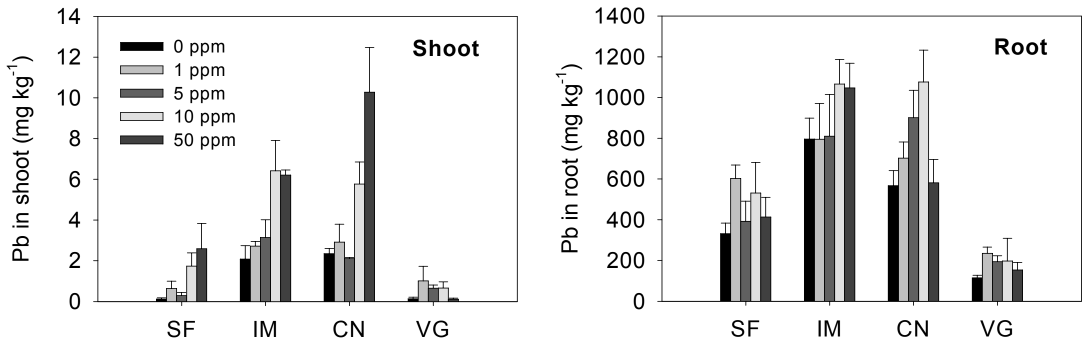

Fig. 4. Lead concentration in shoot and root of plants (sunflower, SF; Indian mustard, IM; canola, CN; vetiver grass, VG) after exposure of gradual citrate concentration in 0.5 mg L^{-1} Pb solution for 24 hours.

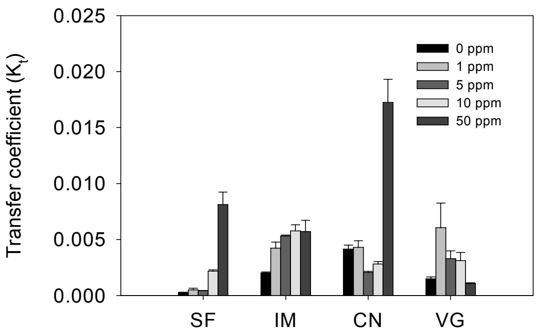

Fig. 5. Transfer coefficient of plants (sunflower, SF; Indian mustard, IM; canola, CN; vetiver grass, VG) after treatment of gradual citrate concentration in 0.5 mg L^{-1} Pb solution.

elevated citrate levels in hydroponic solution is probably due to enhanced adsorption of Pb on the root surface after formation of Pb-citrate complex and enhanced translocation of Pb from root to above ground tissue. As shown in Fig. 5, the transfer coefficient (K_t) of Pb significantly increased for sunflower and Indian mustard indicating more Pb was transferred from root to above ground tissue for these plant species.

Conclusion

It is evident from this study that one of the mechanisms responsible for translocation of Pb from root to shoot in the Indian mustard may be significantly different from that occurring in sunflower and canola which have significantly lower translocation (lower K_t) for Pb than Indian mustard.

While the K_t of Indian mustard increased from 1.94E-3 to 3.93E-3, sunflower and canola showed decreases in K_t as the concentration of Pb increased. Enhanced translocation by Indian mustard seemed to be induced by elevated total organic acids exuded from Indian mustard roots. The concentration of total organic acids exuded from Indian mustard increased from 424 to 6656 mg kg^{-1} even though the total DOC decreased in the higher Pb treated plants. The exudation of organic acids in Indian mustard was significantly different from sunflower and canola. In addition, complexation of Pb with citrate enhanced Pb accumulation in the shoots of all plants studied and caused an increase in K_t implying complexation of Pb with organic acids is involved in the Pb uptake and translocation mechanism. Even though the positive role of organic acid exudation from roots on Pb uptake and translocation were observed in the present study, further experiments using Pb contaminated soil should be conducted in order to ascertain the role of organic acids associated with the soil:root interface because there have been discrepancy between the results by hydroponics and those by pot studies due to the widely different environment.

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