Application of exogenous organic acids and remediation process of lead and cadmium contamination in canola plants

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Abstract

Heavy metal contamination of soil, water and air has caused serious environmental hazard in the biosphere due to rapid industrialization and urbanization. A variety of metal binding ligands such as organic acids and amino acids involve in the heavy metal remediation mechanisms by plants. This paper analyses the possible role of amino acid histidine and organic acids, namely, citric, oxalic and fumaric acids to elevate the absorption of Pb and Cd by canola plants. Soaked seeds were placed in plastic dishes of 10cm diameter with a layer of filter paper on bottom and 10 ml aqueous solution of each treatment with certain Pb and Cd and concentration was added. After 72 hours, the content of Cd and Pb in canola seedlings was measured using atomic absorption technique. All data compared using ANOVA test and SPSS (v. 17) and $p<0.05$. The results showed that oxalic acid and citric acid elevate the accumulation of cadmium in experimental group (plants under Cd treatment with no organic acid exposure). Although all organic acid treatments (including histidine as a mono-carboxylic acid) increase Pb absorption and accumulation by canola plants, Pb accumulation was higher in plants just under Pb treatment with no additional carboxylic acid exposure. Therefore, the possible role of organic acids in heavy metals detoxification and resistance processes by canola plants is suggested for further research.

Keywords: canola; cadmium; lead, phytoremediation; organic acids

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Introduction

Heavy metal pollution is of considerable importance relevant to the present scenario due to the increasing levels of pollution and its obvious impact on human health through the food chain (Aravid and Prasad, 2005). Lead (Pb) is among the most toxic heavy elements in the atmosphere (Fergusson, 1990). Lead is a micro element naturally present in trace amounts in all biological materials, i. e., in soil, water, plants and animals (Strmiskova, 1992). Soil lead contamination is a major environmental problem facing the modern world (Body et al., 1991). It has no physiological function in the organism (Neumann et al., 1990). Sources of Pb contamination in soils can be classified into three broad categories: industrial activities such as mining and smelting processes, agricultural activities such as application of insecticide and

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municipal sewage sludge, and urban activities (Holden, 1989). Lead is absorbed by plants through roots where most of the lead is also accumulated. Excessive lead accumulated in plant tissue can be toxic to most plants, leading to decrease in seed germination, root elongation and biomass (Balsberg, 1989).

Cadmium is a relatively volatile element not essential to plants, animals and humans (Souza and Narurkar, 1994). Its presence in live organisms is unwanted and harmful. An increased level of cadmium in the air, water and soil increases its uptake by live organisms. It is taken up by plants and animals and through them also by humans. This leads to the cadmium cycle soil-plant-animal-man. It has been stated that up to 90% of cadmium taken up by plants originates from soil and only 10% from the atmosphere. Uptake of cadmium by plants occurs through roots and leaves. Plants take up cadmium from water only in the form of Cd^+ ion (after being released from the sorption complex or from soil solution). Additional cadmium is transported to roots by diffusion and mass soil flow (Smirjakova et al., 2005).

Green plants are starting link of the food chain, which is the principal source of cadmium and lead for animals and humans. In order to prevent danger to humans it is necessary to eliminate or at least minimize intentionally and systematically the penetration of extraneous matter into feed and the human food chain (Smirjakova et al., 2005).

Plants respond to heavy metal toxicity in a variety of ways. Such responses include immobilization, exclusion, chelation and compartmentalization of metal ions, and the expression of more general stress response mechanisms such as ethylene and stress proteins. One recurrent general mechanism for heavy metal detoxification in plants and other organisms is the chelation of the metal by ligand and, in some cases, the subsequent compartmentalization of ligand metal complex.

A ubiquitous mechanism for heavy-metal detoxification and accumulation is the chelation of the metal ion by ligand. A variety of metal-binding ligands have been described in plants and their respective roles in heavy metal detoxification have been reviewed. Such ligands include organic acids, amino acid and peptides. When in excess amount, trace elements are mostly achieved through organic acid and histidine. Organic acids, such as fumarate, oxalate and citrate play an important role in several major metabolic pathways in plants such as in fatty acid biosynthesis and oxidation, and carbohydrate biosynthesis . Citrate, oxalate and fumarate are supposed to be responsible for chelation and transportation of Pb and Cd into vacuoles and also take part in the detoxification mechanisms (Jocsak et al., 2005). In this study, we investigated the role of some organic acids in absorption and accumulation of Cd and Pb metals by *Brassica napus* L. seedlings. The main goal of this study was to find out if addition of organic ligands to the environment enhances phytoremediation of metals by canola plants. We addressed this question by carrying out laboratory experiments.

Materials and Methods

Seed of canola (*Brassica napus* L. var. zarpham) was obtained from the Seed Research Center of Karaj, Iran. Seeds were air dried and stored at room temperature before treatments with metal and organic carboxylic acids solution. The germination of the seeds was more than 90%. Seeds were surface sterilized in 5% sodium hypochlorite solution for 10 minutes before use, to avoid fungal contamination. The selected seeds were placed in 10 cm diameter Petri dishes lined with filter paper Whatman No. 42. Each dish received 20 seeds of canola and 20 mL of treatment solution. Lead and cadmium treatments were prepared using lead chloride and cadmium chloride with concentrations 10^{-3} and $500*10^{-6}$ mol/L, respectively. Distilled water was added to the control Petri dishes.

The experiment was conducted in a growth chamber at 25 $^{\circ}$C, 16 hours light /8 hours dark period, (illumination of 2500 lux, Philips T2 40W/33 lamp). This experiment was arranged in a complete randomized block design and each block contained three replicates for each treatment. Seedlings were allowed to grow for 72 hours then they were taken out from the solutions and washed carefully. The measurements were made for Cd and Pb accumulation in canola seedlings.

Atomic absorption

For digestion of samples, 1 g (dry weight) sample was digested with repeated additions of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). The sample was mixed thoroughly to achieve homogeneity and then sieved. For each digestion procedure, 1 g sample (dry weight) weighted to digestion vessel, and 10 ml of 1:1 HNO_3 was added and was covered with a watch glass. The sample was heated to 95 ± 5 $^{\circ}$C and refluxed for 10 to 15 minutes without boiling. The sample was left to cool and then 5 ml of concentrated HNO_3 was added. After replacing the cover, sample was refluxed for 30 minutes. After generating brown fumes, this step (addition of 5 ml of conc. HNO_3) was repeated over and over until no brown fumes was given off by the sample indicating the complete reaction with HNO_3. Using a ribbed watch glass, the solution was allowed to evaporate to approximately 5 ml without boiling for two hours. After the sample was cooled, 2 ml of water and 3 ml of 30% H_2O_2 were added. The vessel was covered with a watch glass and returned to the heat source for warming and to start the peroxide reaction. Heating was continued until the acid-peroxide digestion completed and the volume reduced to approximately 5 ml. After cooling, solution was diluted to 100 ml with water. The obtained solution was then analyzed using GFAA. A spectra-220 atomic adsorption spectrometer and GTA-110 graphite tube atomizer and sample dispenser were used for this study. Deuterium background correction was used. All equipments were from Varian Techron Pty. Limited, Mulgrave, Victoria, Australia.

Statistical Analysis

All analyses were done on a completely randomized design. Data were analyzed by the analysis of variances (ANOVA) using Duncan test. The data were the means of three replicates significance of differences between means were considered at $p<0.05$. All statistical analyses were performed using the SPSS.

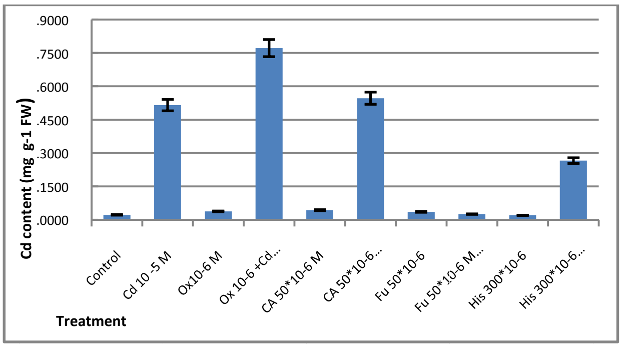

Fig. I. Effect of citric acid (CA), fumaric acid (Fu), oxalic acid (OX) and histidine (His) on cadmium (Cd) absorption by canola seedlings ($p<0.05$)

Results

The results showed that Ox^{-6} + Cd^{-5} treated seedlings accumulate the highest concentration of Cd compared to the other groups. Seedlings under Cd^{-5} stress treated by $50*CA^{-6}$ also showed higher content of Cd compared to Cd experimental group (just under Cd stress). Cadmium stress plants treated by other organic acids showed lower Cd concentration compared to experimental plants (Fig. I). As it is obvious in Fig. II, the highest accumulation of Pb was found in seedlings treated just by Pb (Pb experimental group). There was significant difference between cadmium accumulation in plants treated with different organic carboxylic acids and oxalic and citric acids showed the highest elevating Pb absorption effects, respectively; however, none of the organic acids used in this investigation increased Pb absorption by the canola seedlings, and even they caused lower absorption of Pb by treated

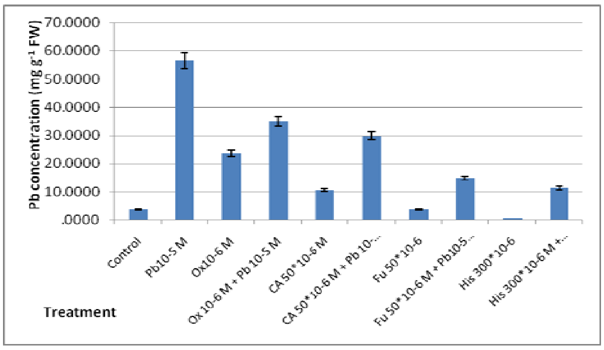

Fig. II. Effect of citric acid (CA), fumaric acid (Fu), oxalic acid (OX) and histidine (His) on lead (Pb) absorption by canola seedlings ($p<0.05$)

seedlings compared to control. Seedlings treated by OX^{-6} showed higher Cd accumulation compared to plants treated with the other organic acid.

Discussion

The result showed that phytoremediation (for cadmium polluted environment) is possible with canola plants treated with adequate concentration of oxalic and citric acids. While for lead the application of these chelators showed no advantage to phytoremediative processes, it was very useful for Cd treatment. However, It seems that oxalic acid has the highest effects in Pb remediation from contaminated medium compared to the other organic acids. Nevertheless, the effects of organic agents in alleviation of Pb toxicity or metals detoxification have to be studied.

There are many controversial reports on the effect of organic ligands on metal absorption by plants which some of them support our findings. Wenger et al., (2000) investigated the influence of natural organic agents, citric, oxalic and salicylic acid and some synthetic organic agents on metal solubilization and absorption by some crop plants. They showed that some organic agents they used were more efficient in solubilizing metal and that in turn caused an increase in metal absorption by the plants. Sun et al., (2005) stated that increasing supply of citric acid increased Cd uptake by wheat roots. The same researchers reported that addition of EDTA, malic acid and oxalic acid decreased Cd concentrations in wheat plants. Studying on different types of organic compounds, these scientists concluded that under various organic chelator-conditions, the degree of Cd absorption and toxicity positively correlated with Cd-induced PCs-SH (phytochelatins, as natural metal ligands in plant cells) synthesis by plants. They also stated that citric acid exhibited stronger effects on decreasing Cd toxicity than malic acid and oxalic acid, accompanied by enhanced uptake of Cd and suppressed transport of Cd to above-ground parts. This is desirable, not only because it alleviates Cd injury, but also it reduces the transportation of Cd into above-ground parts. One possible explanation for this phenomenon is that citric acid forms complexes with Cd, and facilitates Cd uptake from solution to root, and then absorbed Cd-citric complexes stores in inert parts, such as vacuole, resulting in a decrease in the transportation of Cd from root to shoot (Sun et al., 2005).

On the other hand, by root-zone modeling of heavy metal uptake in the presence of organic ligands, Seuntjens et al., (2004) stated that addition or exudation of ligands does not necessarily increase the solubility, transport and bioavailability of metals. Depending on the conditions (mainly the pH), also reduced transport and uptake can be observed, either by formation of ternary surface complexes or reduction of free metal concentration (Seuntjens et al., 2004). By working on radish plants, scientist also showed that citric acid, which forms similar metal complexes as oxalate, reduced uptake of Pb and Cd into the roots (Chen et al., 2003). Ashworth and Alloway (2008) stated that organic- molecules contain functional groups (e.g., COOH and phenolic-OH) capable of binding metals. This process has been implicated in decreasing the sorption and increasing the mobility of heavy metals in soils. This is thought to occur because of the formation of soluble, organic complexes of heavy metals, which tend to resist dissociation and hence (re)adsorption by the soil solid phase (Ashworth and Alloway, 2008).

Duarte et al., (2007) in a work for evaluation of the uptake of nickel and cadmium when supplemented with different concentrations of exogenous citric acid, reported that the application of citric acid with any of the concentrations of nickel leads to a statistically significant decrease in the uptake of nickel. This uptake decreased by approximately 97% with the application of citric acid. They attributed this finding to one of two reasons. One is that citric acid complexation increased the mobility of nickel leading, also, to an excess of nickel mobilization, turning this metal from a nutrient state to a phytotoxic form. This increase of mobility is also directly related to an increase of the solution acidification, caused by the application of citric acid. The other possible reason for this decrease in the uptake of Ni is that the presence of citric acid resulted in the formation of citric acid–nickel complexes that inhibited the uptake. Once again the researchers attributed this to the decrease in the solution pH and consequent increase of mobility, leading to a sustainable increase of the Cd uptake in its less toxic forms (Duarte et al., 2007). Based on the results, it is possible that oxalic and citric acids are capable to form a complex with cadmium but not with lead metal. This complexation resulted in higher cadmium absorption by canola seedlings and therefore higher Cd accumulation in this plant. On the other hand, organic acids used in this study had no significant effect on lead accumulation in canola seedlings. This could partly be attributed to lack of lead complexation with the used organic agents or these agents may need different environmental pH or higher exposure duration.

In this study, the effect of some organic acids including oxalic, citric, fumaric acids and amino acid histidine on cadmium and lead absorption were investigated in 3-day old canola seedlings. Based on the results, particular concentrations of oxalic acid and citric acid elevate cadmium absorption by canola seedlings compared to experimental plants (plants under cadmium treatment with no organic compound) while none of the organic treatments caused higher lead accumulation compared to control and experimental plants (plants under lead treatment with no organic compound). None of the organic acids used in this investigation increased Pb absorption by the canola seedlings and they even lowered absorption of Pb by treated seedlings compared to control. Seedlings treated with OX^{-6} showed higher Cd accumulation as compared to the other organic acid treated plants.

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