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CITRIC ACID MEDIATED PHYTOEXTRACTION OF CADMIUM BY MAIZE (*ZEA MAYS* L.)

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Abstract

The aim of the investigation was to determine the potential of citric acid for accumulation and translocation of cadmium and their effect on maize growth. The plants were grown in small plastic glasses and treated with 300 mg kg^{-1} $CdCl_2$ and 0, 0.25, 0.5, 1 and 2 g kg^{-1} of citric acid. After 10 days, the plants were harvested, dried and root and shoot biomass weighed. To study the efficiency of maize to bioaccumulate metal, uptake of cadmium was studied in the root and shoot. The results showed that heavy metal accumulated more in roots than the shoots and application of citric acid depressed Cd uptake at all concentrations. Percent decrease in Cd uptake was 58, 35, 26, 25 and 63, 46, 44, 42 by Sahiwal-2002 and Pak-affgoee, respectively at 0.25, 0.5, 1 and 2 g kg^{-1} of citric acid application. Maize proved to be an effective accumulator for cadmium, however, neither concentration of citric acid showed advantages for phytoextraction of cadmium.

Introduction

Burning oil, automobiles, tire dust, cadmium batteries, electroplating, polyvinyl plastic, canned foods, silver polish residue on eating utensils, metal ice trays, processed meats, pottery, plastic wrappings and the incineration of municipal waste are the main sources of cadmium resulting in buildup of cadmium in soil, air and living organisms (Anon., 1997). Cadmium is non-essential toxic heavy metal and relatively mobile, thus easily transferred to food chain from polluted agricultural land (Xiao *et al.*, 2008; Chakroun *et al*., 2010; Ahmad *et al*., 2011; Mojiri, 2011).

Phytoextraction is an environment friendly technique to remove heavy metals from contaminated soil or water, which involves the extraction of metals by plant roots and then translocation to the shoots (Islam *et al*., 2007; Achakzai *et al*., 2011)). Induced phytoextraction is the use of some metal-chelates and high biomass crop species to accumulate and remove heavy metals from the soils. Synthetic chelates proved efficient for phytoextraction; however have a drawback of leaching and ground water contamination. Citric acid is cost effective and environment friendly chelate for use in phytoextraction without a risk of leaching and ground water contamination due to the rapid degradation (Melo *et al*., 2008; Wuana, *et al*., 2010). Several researchers have reported that citric acid proved beneficial for mobilization and phytoextraction of Cd (Turgut *et al*., 2004; Sinhal *et al*., 2010). A plant used for induced phytoextraction should have high biomass, tolerant to metal stress and preferably capable of producing usable fruit or yield, thus giving some financial benefits after harvesting (Robinson *et al*., 2000; Elkhatib *et al.*, 2001; Mukhtar *et al*., 2010). Maize has high potential to tolerate and extract Cd from soil (Mojiri, 2011). Thus, the study was undertaken to find out the potential of citric acid and determining its optimum concentration for mediating the Cd extraction by maize.

Materials and Methods

Seeds of two maize (*Zea mays* L.) varieties viz., Sahiwal-2002 and Pak-affgoee obtained from Maize and Millet Research Institute Yousafwala, Sahiwal, Pakistan were sown in small plastic cups filled with sand. Treatment solution contained 0 and 300 mg kg^{-1} $CdCl_2$ with various concentrations of citric acid (0, 0.25, 0.5, 1 and 2 g kg^{-1}).

The plants were harvested after 10 days and roots were washed with 5 m*M* ice cold $CaCl_2$ solution to remove extracellular Cd (Rauser, 1987) and then treated with deionized water. Both roots and shoots were excised, blotted with filter paper and weighed. All samples were then oven dried at 70ºC and digested with concentrated H_2SO_4 and H_2O_2. The concentration of Cd in shoots and roots was determined using Atomic Absorption Spectrophotometer.

Translocation factor is the ability of plant to translocate heavy metal from roots to the shoot and was determined according to the formula devised by Anamika *et al*. (2009).

$$\text{Translocation factor (TF)} = \frac{\text{Shoot metal content}}{\text{Root metal content}} \times 100$$

Bioaccumulation coefficient was calculated as element concentration in plant part divided by the concentration of that element in the external medium (Anamika *et al*., 2009; Liao & Chang, 2004).

$$\text{Bioaccumulation coefficient (BC)} = \frac{\text{Cadmium content mg g}^{-1}\text{ dry plant tissue}}{\text{Cadmium content mg ml}^{-1}\text{ nutrient solution}}$$

Results and Discussion

Metal tolerance: Significant inhibitory effect of Cd contamination was determined on various growth attributes such as shoot, root length, fresh and dry biomass (Figs. 1, 2). All of these parameters showed significant reduction under Cd stress. Kabir *et al*. (2008) found similar reduction at 10-70µmol/L of Cd.

Cadmium at 300 mg kg^{-1} caused 56 and 52 percent reduction in shoot biomass and 60 and 59% reduction in root length of Sahiwal-2002 and Pak-affgoee respectively. Similarly 60 and 53% reduction in shoot fresh biomass, 51 and 46 in root fresh biomass, 45 and 36 in shoot dry mass, 43 and 33 in root dry mass were recorded in Sahiwal-2002 and Pak-affgoee respectively. Sahiwal-2002 exhibited more reduction as compared to the other variety. Cadmium inhibited the root growth more than shoot, which is in line with the finding of Munzuroglu & Zengin (2006), Yasar & Ahmet (2006) and Anamika *et al*., (2009). The reduction in root length was due to more accumulation of metal in root than shoot that resulted in reduction of mitotic cell division by blocking the metaphase in meristematic zone (Aidid & Okamoto, 1992). Cadmium retention was more in roots and less translocation to the shoots, which may be attributed to the Cd tolerance of plant.

Fig. 1a. Effect of different citric acid levels (2, 1, 0.5, 0.25,0 mg/L) on growth of Sahiwal-2002.

Fig. 1b. Effect of different citric acid levels (2, 1, 0.5, 0.25,0 mg/L) on growth of Pak-affgoee.

Fig. 1c. Effect of different citric acid levels (2, 1, 0.5, 0.25,0 mg/L) +300mg/L Cd on growth of Sahiwal-2002.

Fig. 1d. Effect of different citric acid levels (2, 1, 0.5, 0.25,0 mg/L) +300mg/L Cd on growth of Pak-affgoee.

Citric acid effect on plant growth: Citric acid when applied alone resulted in gradual increase in shoot length of Sahiwal-2002 up to 1 g $kg^{-1}$of citric acid (24.41 cm) as compared to control (16.05 cm). Maximum root length (16.77 cm) was observed at 0.25 g kg^{-1}, whereas fresh and dry biomass enhanced at 0.5 g kg^{-1} citric acid. In Pak-affgoee cv., shoot length increased at 0.25 g $kg^{-1}$and then gradually decreased at high concentrations. Muhammad *et al*., (2009) noted similar increase in root dry weight at 2.5 and 5 m*M* citric acid application (Fig. 2). Thus, citric acid at low concentration improved plant biomass by mobilizing the weakly soluble essential nutrients (Strom *et al.*, 2001).

Citric acid alleviated the toxic effect of Cd on root and shoot biomass. Application of 0.5 g kg^{-1} of citric acid showed maximum improvement in plant biomass (Table 1). Previous studies also reported that citric acid detoxified Cd and Pb (Chen *et al*., 2003), Mn (Najeeb *et al*., 2009), Al (Ginting *et al*., 2000) and salinity stress (Sun & Hong, 2010) by chelation, either externally in the soil or internally by plant roots (Ma, 2000).

Cadmium accumulation/ uptake: Sahiwal-2002 accumulated more Cd (76.67 in shoot and 206 mg kg^{-1} in roots) as compared to Pak-affgoee (36 in shoot and 124.3 mg kg^{-1} in roots). Cadmium accumulated more in roots than in shoot, which showed that roots of maize are more effective in Cd extraction. Retention of Cd in roots of maize and limited translocation to the shoot can be attributed to Cd tolerance of the plants (Bavi *et al*., 2006). This is also in line with findings of Jadia & Fulekar (2008), Xiao *et al*., (2008), Anamika *et al*. (2009), , Aisien *et al*., (2010), Mojiri (2011).

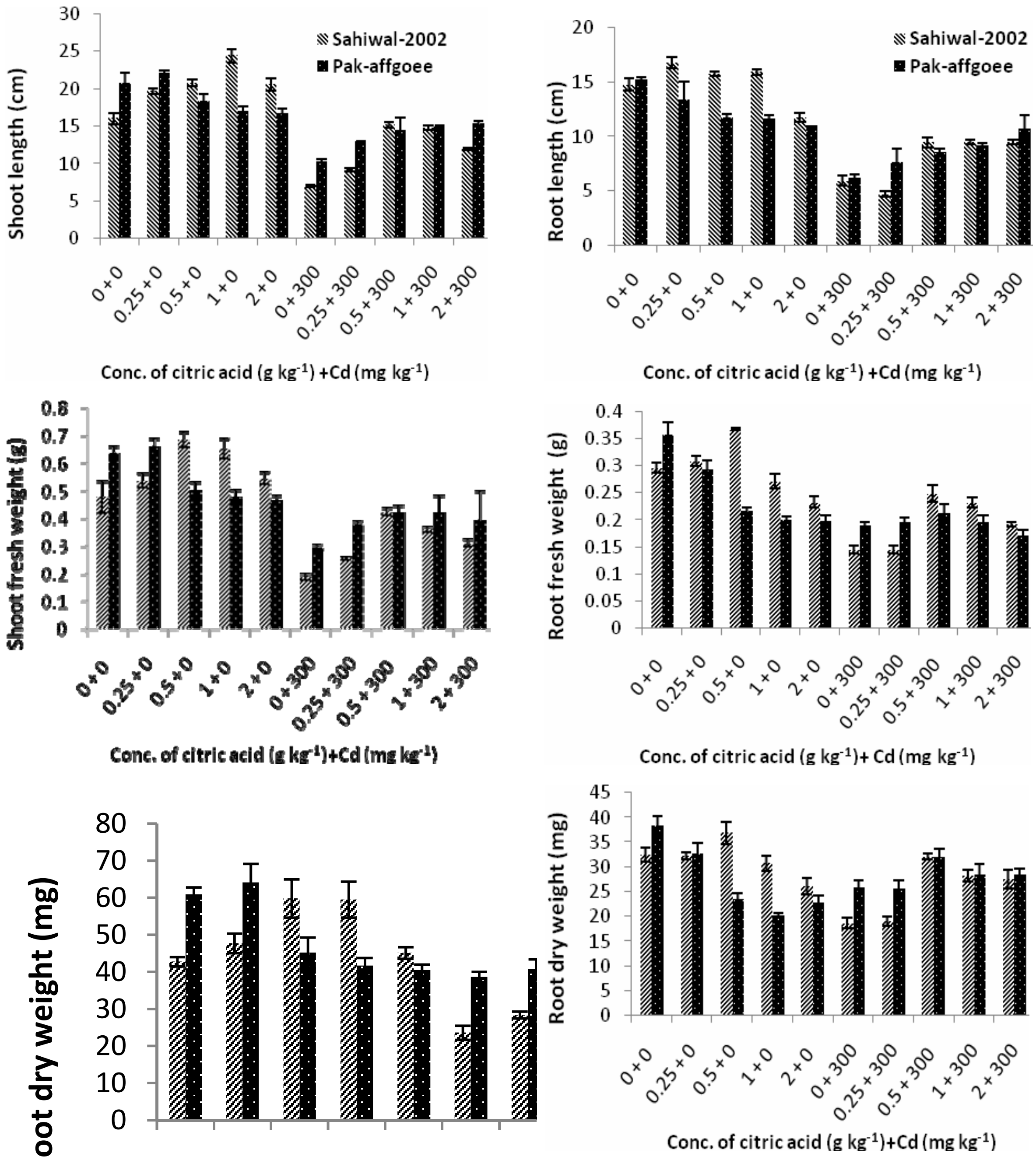

Fig. 2. Effect of induced Cd and citric acid levels on some growth attributes of two maize varieties.

Table 1. Analysis of variance for two maize varieties under different concentrations of Cd and citric acid.

Source	df	Mean squares							
		Shoot length(cm)	Root length(cm)	Shoot fresh weight(g)	Root fresh weight(g)	Shoot dry weight(mg)	Root dry weight(mg)	Shoot Cd (mg kg^{-1})	Root Cd (mg kg^{-1})
Varieties	1	1.06 **ns**	12.51***	0.007 *	0.0061 ***	1050.01***	4.70 **ns**	9.6 ***	9095.86 ***
Cadmium	1	744.27 ***	472.42***	0.72***	0.098 ***	11979.41***	141.07***	122.92 ***	212849.5 ***
Citric acid	4	33.98***	2.50***	0.02***	0.007 ***	837.99 ***	43.73 ***	8.03 ***	5721.5 ***
Interaction									
Var * Cd	1	38.98***	34.38 ***	0.041 ***	0.007 ***	1135.35 ***	190.10 ***	10.42 ***	9286.5 ***
Var * citric acid	4	28.78***	5.52***	0.031***	0.009 ***	162.27 **	95.24 ***	1.76 **	970.6 **
Cd*citric acid	4	20.53 ***	31.66 ***	0.016 ***	0.011 ***	848.86 ***	191.27 ***	8.13***	5737.7 ***
Var *Cd* citric acid	4	12.68 ***	3.78***	0.011 ***	0.0018 **	113.81**	17.55*	164.44**	941.3 **
Error	40	0.59	0.311	0.001	3.46e-4	24.27	6.40	37.59	222.6
Total	**59**								

Influence of citric acid on cadmium accumulation: Cadmium (Cd) uptake by roots was up to 206 and 124.3 mg kg^{-1} in Sahiwal-2002 and Pak-affgoee varieties respectively (Table 1). The application of citric acid significantly decreased Cd uptake by plants. Percent decrease in uptake was 83.9, 64.1, 54.4 and 48.7 in Sahiwal-2002 with increasing citric acid concentration. Slight increase was observed at 0.25 mg kg^{-1} in Pak-affgoee, which then showed 84.2, 83.4 and 70.8% reduction at higher concentrations. Shoot Cd uptake was 76.7 and 36 mg kg^{-1} in Sahiwal-2002 and Pak-affgoee respectively without citric acid addition. Percent decrease in Cd uptake was 58.26, 35.2, 26.5, 25.2 in Sahiwal-2002, and 62.97, 46.3, 44.4, 41.7 in Pak-affgoee, respectively at 0.25, 0.5, 1 and 2 mg kg^{-1}of citric acid levels (Fig. 3). Lin *et al*., (2001) reported similar Cd uptake reduction by root and shoot, in the presence of citric acid. Reduced uptake might be due to the reason that Cd is highly soluble and bioavailable (Arabi *et al*., 2011), whereas citric acid might transform the exchangeable Cd to relatively stable organic and residual forms (Mojiri, 2011).

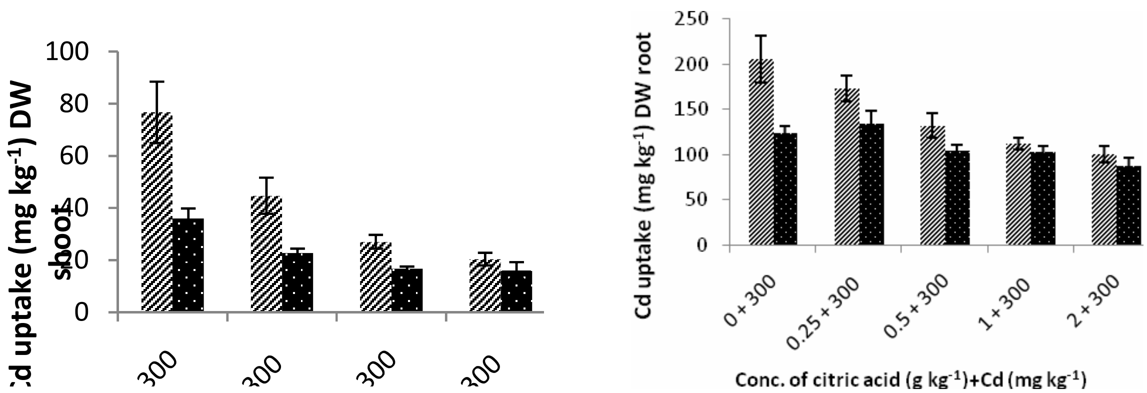

Fig. 3. Cd uptake by root and shoot at different citric acid levels.

Previous studies have shown that organic acids such as citric acid proved less effective for mobilization and phytoextraction of metal as compared to synthetic chelators (Lombi *et al*., 2001; Melo *et al*., 2008) as natural organic acid usually chelate and solubilize essential nutrients, instead of heavy metals (Nascimento, 2006). The negative effect of citric acid on Cd uptake might be due to its rapid degradation (Ström *et al*., 2001). Jia *et al*. (2009) examined that 20% of citric acid degraded between 1 and 4 days, and further 70% degradation within 20 days. Mineralization of citric acid by soil microorganisms (Lesage *et al*., 2005) and/or its re-precipitation and re-adsorption of metal on soil might also be the reasons (Nascimento, 2006). Citric acid less efficiently desorbs soil Cd (Elkhatib *et al*., 2001). Turgut *et al*. (2004) observed that low concentrations of citric acid (0.1 and 0.3 g/kg) were unsuccessful in enhancing the Cd uptake, whereas higher concentration showed severe phytotoxic symptoms with reduced uptake. Contrary to that, Duarte *et al*. (2007) reported that the 25 and 50 µ*M* of citric acid enhanced the uptake of Cd in shoots and roots by *Halimione portulacoides.*

Bioaccumulation coefficient and translocation factor (BCF): Bioaccumulation coefficient (BC) and translocation factor (TF) were used to evaluate the potential of maize to remediate Cd from soil and its translocation from root to shoot. Addition of citric acid reduced this factor from 0.94 (control) up to 0.73, 0.53, 0.44, 0.4 in Sahiwal-2002 and from 0.534 in control to 0.52, 0.40, 0.4, 0.34 in Pak-affgoee at 0.25, 0.5, 1 and 2 g kg^{-1} citric acid respectively (Fig. 4) while, BCF values should be greater than 1 for feasible phytoextraction. Low BCF values indicated that citric acid is inefficient for induced phytoextraction. BCF of Cd increased with growth period of plant and decreased with increasing metal concentration in soil (Han *et al*., 2005; Niu *et al*., 2011). In the present studies, Cd concentration in soil (300 mg kg^{-1}) might be the possible reason for low BCF. In contrary to the present studies, Qu *et al*. (2011) reported that application of sodium hydrogen phosphate/ citric acid increased BCF for Cd by 0.94 and 1.37 for shoot and roots of alfalfa.

TF values showed the percent of root Cd, translocated to the shoot and was 37 and 29 in Sahiwal-2002 and Pak-affgoee respectively (Fig. 5), which then decreased with citric acid application. Maximum decrease (18 in Sahiwal-2002 and 15% in Pak-affgoee) was observed at 1g kg^{-1} citric acid level. Similar decline of TF values of Cd was observed by Qu *et al*. (2011), after sodium hydrogen phosphate/citric acid application.

Conclusion

Maize is relatively tolerant to Cd stress as it showed limited toxicity symptoms at relatively high Cd concentration. Two varieties showed different response to Cd accumulation as Sahiwal-2002 exhibited BCF≈1, whereas Pak-affgoee had BCF<1 at 300 mg kg^{-1} of Cd. Pak-affgoee accumulated less Cd in root and shoot and thus can more likely behave as an excluder. Citric acid proved inefficient for Cd phytoextraction however ameliorated the toxicity of Cd.

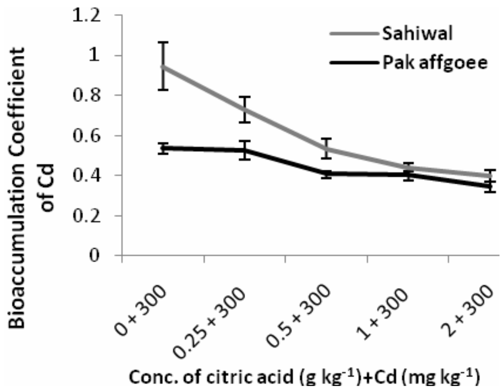

Fig. 4. Bioaccumulation of Cd in plant parts from medium having different citric acid levels.

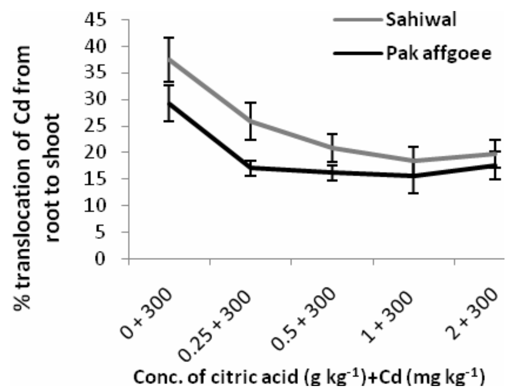

Fig. 5. Changes in Cd translocation factor of two maize varieties at different concentrations of citric acid.

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