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## A Comparative Study on the Organic Acid Content and Exudation in Maize (*Zea mays* L.) Seedlings under Conditions of Copper and Cadmium Stress

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**Abstract:** This study focuses on the comparative effects of copper and cadmium on the organic acid content and exudation in maize (*Zea mays* L.). We have also discussed the role of organic acids in copper and cadmium tolerance. The maize seedlings were grown in hydroponics for 8 days and then treated with 100 µM copper or cadmium for 4 days. The copper reduced more than cadmium root fresh wt and shoot dry wt, while Cd had more decreasing effect on root dry wt. Analysis of organic acids in root exudates showed that citrate exudation was not affected by both metals, suggesting that root exudation was not involved in copper or cadmium tolerance in maize. This prompted us to investigate the role of organic acids in internal tolerance mechanism. Total organic acids were decreased in roots but remained unchanged in shoots of Cu and Cd-treated plants. However, malate content decreased substantially in roots by 68 and 53%, respectively with Cu and Cd. The marked increase in the content of citrate with both metals in roots, as well as in those of malate with Cu and citrate with Cd in shoots, underlined the main role of these organic acids in Cu and Cd tolerance in maize.

**Key words:** Cadmium, citrate exudation, copper, organic acids, maize (*Zea mays*), malate dehydrogenase

### INTRODUCTION

In plants, a number of heavy metals such as zinc and copper are essential for normal growth as cofactors and as structural and catalytic components of proteins and enzymes. However, these micronutrients, as well as nonessential heavy metals such as cadmium, become stressful at high levels in the plant's environment (Tomsett and Thurman, 1988). Heavy metals contamination of the environment can occur in some natural soils or as the result of environmental pollution from mining, smelting, manufacturing, agricultural or waste disposal technologies (Foy *et al.*, 1978). These toxic pollutants affect plant growth at least by inducing the oxidation of molecules catalyzed by metals. Copper has an irreplaceable role as nutritional element in plants and as redox component required for a wide variety of processes including the detoxification of free radicals, lignification of plant cell walls, photosynthesis and electron transfer reactions of respiration (Fox and Guerinot, 1998). It is a cofactor of numerous metallo-enzymes such as cytochrome c oxidase, Cu, Zn-superoxide dismutase (SOD) and several ATPases implied in catalyzing redox reactions (Gupta, 1979). Excess of copper can cause oxidative damage by reacting with oxygen and producing toxic-free radicals (Fenton

reaction). Moreover, copper inhibited numerous enzymes implied in the dark reaction of photosynthesis, particularly Rubisco (Stiborova *et al.*, 1986a). Cadmium a non-essential element is strongly phytotoxic (Clijsters and van Assche, 1985; Sanità di Toppi and Gabbrielli, 1999). In the environment, cadmium is spilled extensively, present by the powerhouses, the heating systems, the metallurgic industries, the incinerators of waste, the urban traffic, the cement industrial unit and present as impurity in the phosphate fertilizers (Sanità di Toppi and Gabbrielli, 1999). Cadmium can cause an increase in lipid peroxidation and lipoxygenase activity (Gallego *et al.*, 1996). Excess cadmium has been also shown to enhance leaf senescence (Gallego *et al.*, 1996). Plants respond to heavy metal stress in a variety of ways, by exclusion mechanism avoiding the toxic effects of the metal, or by internal tolerance mechanisms enabling the plant to survive (Tomsett and Thurman, 1988). Generally, tolerance mechanisms in plants can be divided into (1) metal binding to cell wall, (2) reduced transport across the cell membrane, (3) active efflux, (4) compartmentalization and, (5) chelation of the metal by proteins, organic acids or inorganic compounds (Tomsett and Thurman, 1988). The principle classes of intracellular metal-binding ligands are organic acids, amino acids, phytochelatin (PCs) and metallothioneins (MTs). Organic acids, such as malate

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and citrate, can form complexes with metals in the vacuole (Godbold *et al.*, 1984). Nevertheless, few recent data are available about the role of organic acids in copper and cadmium tolerance. This research was a comparative study on the effects of copper and cadmium on organic acid content and exudation in maize (*Zea mays* L.). The role of organic acids in copper and cadmium tolerance was discussed. Metal effects on the growth and metal accumulation will be presented.

## MATERIALS AND METHODS

**Growth conditions and metal analysis:** Maize (*Zea mays* L.; var. LG 23/01) seeds were surface sterilized with 10% (v/v) H<sub>2</sub>O<sub>2</sub> for 20 min, rinsed many times with tap water and germinated on filter paper moistened with distilled water in the dark, at 25°C for 3 day. The germinated seedlings were transferred to 12l basal nutrient solutions for 4 days. Selected plants of uniform size were selected and then transferred to identical solutions in 6l plastic pots (12 plants each) for 4 days. For treatment purposes, eight-day-old seedlings were transferred to solutions that contain 0 (control), 100 μM CuSO<sub>4</sub> or Cd(NO<sub>3</sub>)<sub>2</sub> for 4 days. Plants were grown hydroponically according to Chaffai *et al.* (2005). At harvest, the roots were rinsed three times with distilled water; the fresh wt was determined and frozen in liquid N<sub>2</sub>. The metal content was determined in roots and shoots and in two fractions: root cell wall and soluble fractions. The root cell wall was isolated by 1% (v/v) Triton X-100 as previously described (Chaffai *et al.*, 2005). The metal content was determined by an atomic absorption spectrophotometer (Perkin Elmer-model 2380).

**Organic acid extraction and HPLC analysis:** Preparation of organic acid extracts of plant tissue and their subsequent separation by high performance liquid chromatography (HPLC) on a Dupont Zorbax SAX-5 μm column (250×4.6 mm, HP) was according to the procedure described previously by Chaffai and El Ferjani (2005).

**Determination of MDH activity:** Frozen material was homogenized in 20 mM Tris-HCl buffer (pH 8.0) containing 5 mM ascorbate. The homogenate was centrifuged at 25000 g for 20 min (4°C). The malate dehydrogenase (MDH, EC 1.1.1.37) activity was determined in protein extracts as previously described (Chaffai and El Ferjani, 2005). The protein was determined by the Bradford (1976) method using BSA as a standard.

## RESULTS

**Effects of Cu and Cd on growth:** After exposure to 100 μM Cu or Cd for 4 days, maize plants showed a significant decrease in root and shoot growth. The root fresh wt was decreased by 54.5 and 32.5% in the presence respectively of excess Cu and Cd in solution; however, shoot fresh wt was similarly decreased by 31.2% (Fig. 1A and B). The Cd decreased by 42.7% and Cu by 23.6% the root dry wt. However, Cu and Cd decreased the shoot dry wt, respectively by 49 and 24.5% (Fig. 1C and D).

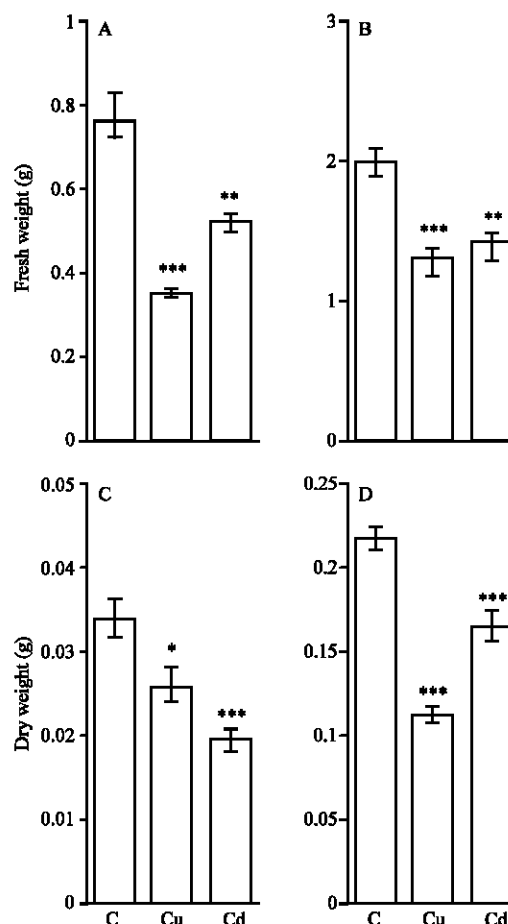


Fig. 1: Effects of copper and cadmium on root (A, C) and shoot (B, D) fresh and dry weight of maize (*Zea mays* L.). The seedlings were grown in nutrient solutions containing 100 μM Cu or Cd for 4 days. Histograms represent means±SE (n = 6) of six separate experiments. One Way ANOVA is performed for comparison between control and treated plants. Asterisks show statically different means between control and treated plants: \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001

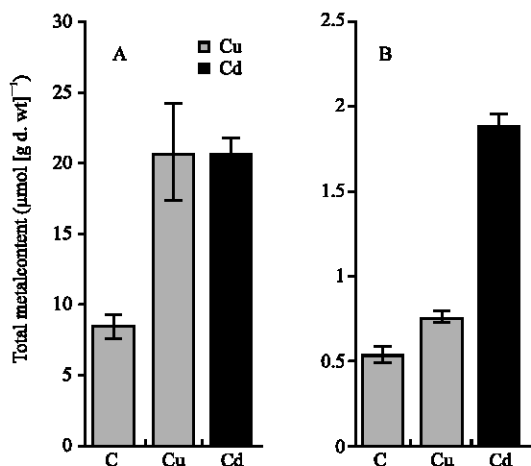


Fig. 2: Total content of copper and cadmium in roots (A) and shoots (B) of maize (*Zea mays* L.). The seedlings were grown in nutrient solutions at 100 µM Cu or Cd for 4 days. Histograms represent means±SE (n = 4) of four separate experiments

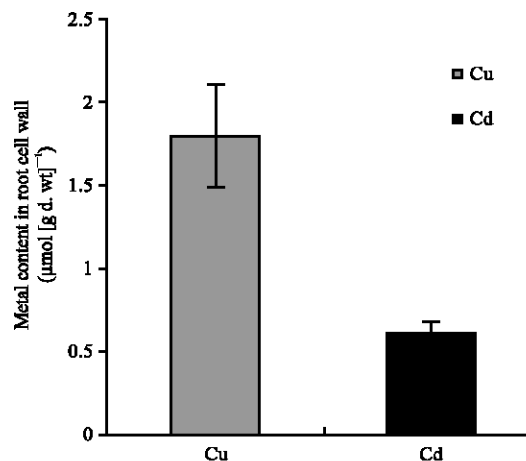


Fig. 4: Cell wall fractions of copper and cadmium in roots of Cu and Cd-treated plants. The seedlings were grown in nutrient solutions at 100 µM Cu or Cd for 4 days. Histograms represent means±SE (n = 6) of six separate experiments.

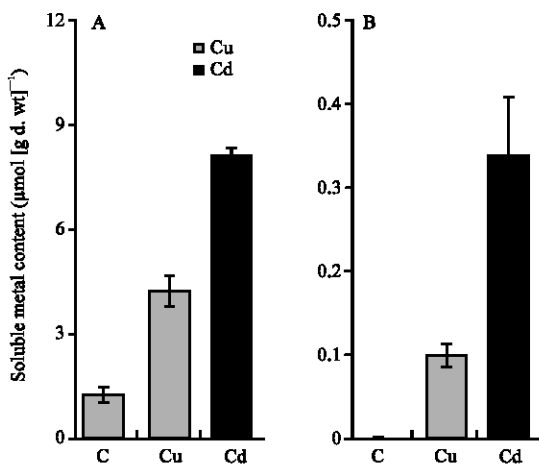


Fig. 3: Soluble fractions of copper and cadmium in roots (A) and shoots (B) of maize (*Zea mays* L.). The seedlings were grown in nutrient solutions at 100 µM Cu or Cd for 4 days. Histograms represent means±SE (n = 4) of four separate experiments

**Cu and Cd content:** The results showed that Cu concentrations in roots of maize plants grown at 100 µM Cu were 27-fold higher than those in shoots; however, Cd concentrations were 11-fold higher (Fig. 2). The total concentrations of Cd in roots of Cd-treated plants were similar to those of Cu in roots of Cu-treated ones (Fig. 2A). In contrast, the Cd concentrations in shoots were 2.5-fold higher than Cu in shoots of Cu-treated plants (Fig. 2B). The soluble fraction of both metals was greater in roots than in shoots (Fig. 3). The soluble fraction of Cu was 41.5-fold greater in roots than in shoots; however that of Cd was 23.7-fold greater (Fig. 3). The soluble fraction of Cu represented 20.1% of total Cu content and that of Cd 37.2% of total Cd content of maize treated plants. With regard to cell wall fraction, the Cu in root cell walls in the presence of 100 µM Cu, was 3-fold greater than Cd in root cell walls in the presence of 100 µM Cd. This fraction represented 8.3 and 2.7% of the total metal content of roots treated with Cu and Cd respectively (Fig. 4).

Table 1: Effects of Cu and Cd on organic acid concentrations in roots of maize (*Zea mays* L.). The seedlings were grown in nutrient solutions containing 100 µM CuSO<sub>4</sub> or Cd(NO<sub>3</sub>)<sub>2</sub> for 4 days

Organic acid concentrations in roots (µmol [g fresh wt] <sup>-1</sup> )						
	Succinate	Malate	Citrate	Tartarate	Lactate	Total
C	15.94±1.36a	137.98±24.03a	19.41±1.26a	761.66±26.48a	6.10±0.90ab	975.95±58.96a
Cu	13.04±2.12a	44.61±12.25b**	29.68±2.33b*	589.21±39.72b**	4.35±1.20a	669.70±55.79b**
Cd	21.78±1.59b*	65.21±13.61b*	30.18±2.04b**	382.40±28.67b***	14.42±2.84bc	509.08±14.95b***

Values represent means±SE (n = 5) of five separate experiments. One way ANOVA is performed for comparison between control and treated plants. Different letters within the same column indicate significant differences. Asterisks show statically different means between control and treated plants: \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001. C, control

Table 2: Effects of Cu and Cd on organic acid concentrations in maize (*Zea mays* L.) shoots. The seedlings were grown in nutrient solutions containing 100  $\mu$ M CuSO<sub>4</sub> or Cd(NO<sub>3</sub>)<sub>2</sub> for 4 days

Organic acid concentrations in shoots ( $\mu$ mol [g fresh wt] <sup>-1</sup> )							
	Succinate	Malate	Citrate	Tartarate	Lactate	Salicylate	Total
C	24.36±4.70a	254.03±43.47a	31.66±0.76a	811.88±31.37a	416.26±84.86a	nd	1286.98±167.84a
Cu	29.24±4.10a	478.14±59.82b*	43.58±1.95a	618.73±16.19b**	361.00±99.32a	0.19±0.02a	1336.22±123.54a
Cd	21.19±3.92a	376.23±86.10a	61.64±5.20b**	662.39±23.76b**	350.42±59.20a	0.24±0.03a	1330.60±90.08a

Values represent means±SE (n = 5) of five separate experiments. One way ANOVA is performed for comparison between control and treated plants. Different letters within the same column indicate significant differences. Asterisks show statically different means between control and treated plants: \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001. C, control; nd, not detected

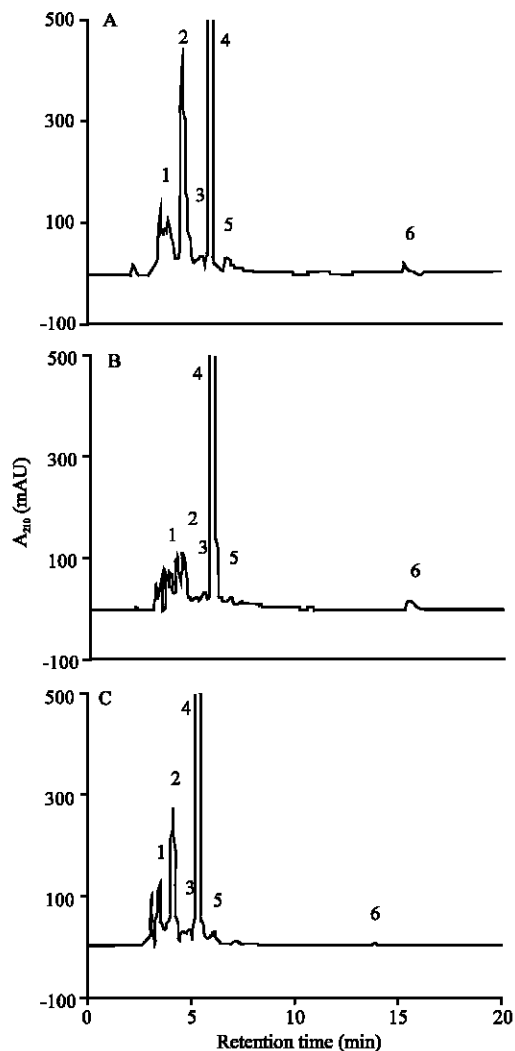


Fig. 5: HPLC chromatograms of organic acids in root extracts of maize (*Zea mays* L.). The seedlings were grown in nutrient solutions containing 0 (A); 100  $\mu$ M Cu (B) or Cd (C) for 4 days. The organic acids were separated on a 250×4.6 mm i.d. HP Zorbax SAX, a strong anion exchange column. The mobile phase consisted of 0.2 M KH<sub>2</sub>PO<sub>4</sub> (pH 3.0) at a flow rate of 1 mL min<sup>-1</sup> and at 25°C. The elution peaks: 1, succinate; 2, malate; 3, citrate; 4, tartarate; 5, lactate; 6, unknown

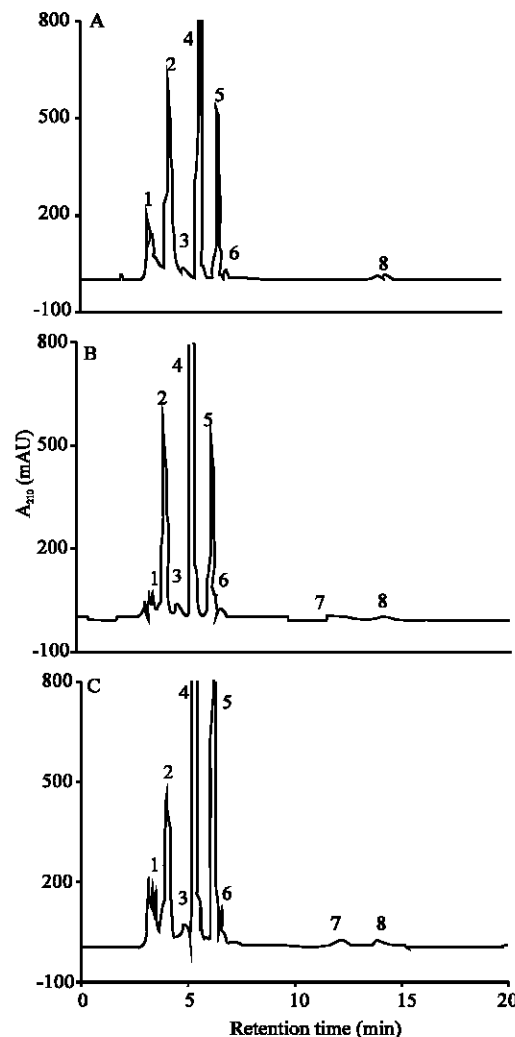


Fig. 6: HPLC chromatograms of organic acids in shoot extracts of maize (*Zea mays* L.). The seedlings were grown in nutrient solutions containing 0 (A); 100  $\mu$ M Cu (B) or 100  $\mu$ M Cd (C) for 4 days. The organic acids were separated on a 250×4.6 mm i.d. HP Zorbax SAX, a strong anion exchange column. The mobile phase consisted of 0.2 M KH<sub>2</sub>PO<sub>4</sub> (pH 3.0) at a flow rate of 1 mL min<sup>-1</sup> and at 25°C. The elution peaks: 1, succinate; 2, malate; 3, citrate; 4, tartarate; 5, lactate; 6, unknown; 7, salicylate; 8, unknown

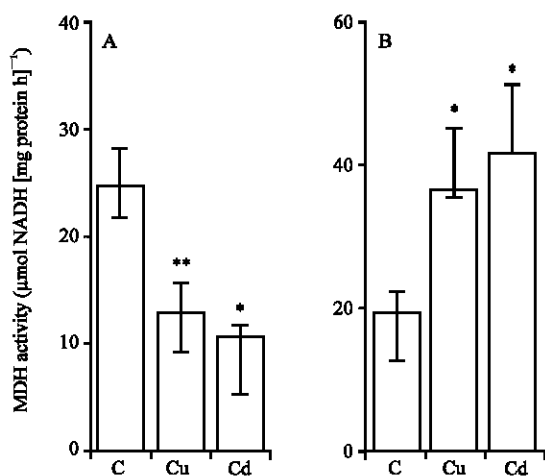


Fig. 7: Effects of copper and cadmium treatment on MDH activity in roots (A) and shoots (B) of maize (*Zea mays* L.). The seedlings were treated with 100  $\mu$ M Cu or Cd for 4 days. Histograms are means $\pm$ SE (n = 6) of six independent experiments. Asterisks show statically different means between control and treated plants: \*, p<0.05; \*\*, p<0.01

**Organic acid content in roots:** Five organic acids in root extract from control and Cu and Cd-treated plants were identified by comparing their retention time (Rt) with those of known standards. Organic acids found were succinate (peak 1, Rt 3.80), malate (peak 2, Rt 4.08), citrate (peak 3, Rt 4.57), tartarate (peak 4, Rt 5.31) and lactate (peak 5, Rt 6.11). A non identified peak (peak 6) was detected with Rt of 13.92 min (Fig. 5). The organic acid analysis showed that total organic acids in roots decreased significantly with both metals (Table 1). The decrease was to 31.4 and 47.8%, respectively with Cu and Cd. Both metals decreased markedly the malate content by 67.7 and 52.7%, respectively with Cu and Cd. In the same way, the tartarate content decreased significantly by 22.6 and 49.8% with Cu and Cd (Table 1). However, citrate content increased substantially by 52.9 and 55.5% respectively with Cu and Cd. The succinate content showed a lesser increase only by Cd. Moreover, lactate content of the roots did not change under Cu or Cd treatment (Table 1).

**Organic acid content in shoots:** In the chromatograms of organic acids in shoot extracts, 6 peaks were identified corresponding to succinate (peak 1, Rt 3.52), malate (peak 2, Rt 3.98), citrate (peak 3, Rt 4.63), tartarate (peak 4, Rt 5.36), lactate (peak 5, Rt 6.19) and salicylate (peak 7, Rt 12.19). In addition to these peaks, the chromatograms revealed two other unidentified peaks with Rt 6.64 (peak 6) and Rt 14.08 (peak 8), respectively (Fig. 6). Exposure to 100  $\mu$ M Cu or Cd had no significant

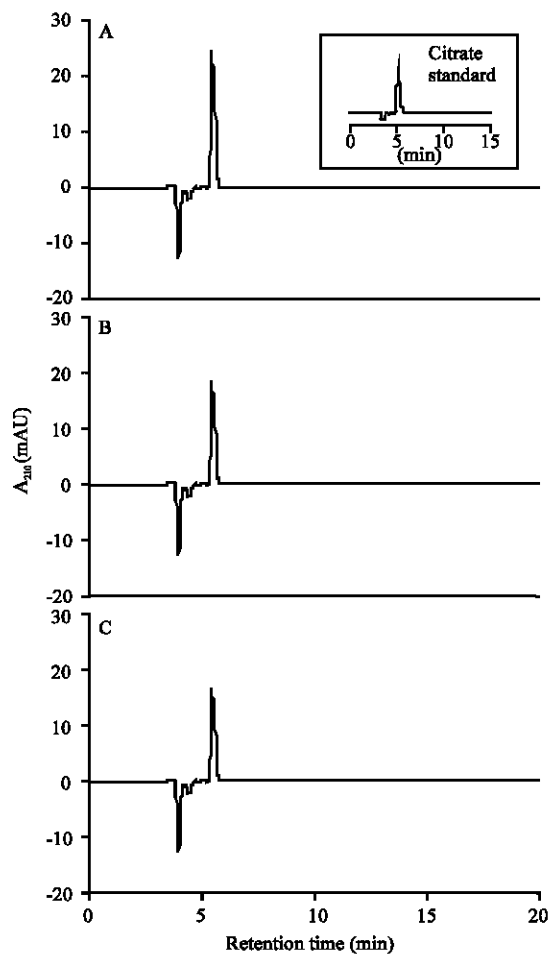


Fig. 8: HPLC chromatograms of organic acids in root exudates of maize (*Zea mays* L.). The seedlings were grown in nutrient solutions containing 0 (A); 100  $\mu$ M Cu (B) or Cd (C) for 4 days. The organic acids were separated on a 250 $\times$ 4.6 mm i.d. HP Zorbax SAX, a strong anion exchange column, using 0.2 M  $\text{KH}_2\text{PO}_4$  (pH 3.0) as mobile phase at a flow rate of 1 mL  $\text{min}^{-1}$  and at 25 $^\circ\text{C}$

effect on total organic acid content in shoots (Table 2). Similarly, the succinate and the lactate content were not affected with Cu and Cd treatments. However, the tartarate content was decreased with both metals (Table 2). By contrast, the malate and citrate content increased 1.9-fold, respectively with Cu and Cd (Table 2). In addition, salicylic acid was detected in shoots of treated plants but not in control plants (Table 2).

**MDH activity:** A marked decrease in MDH activity was observed in roots of Cu and Cd-treated plants (Fig. 7A). The activity of this enzyme was decreased by 47.8 and

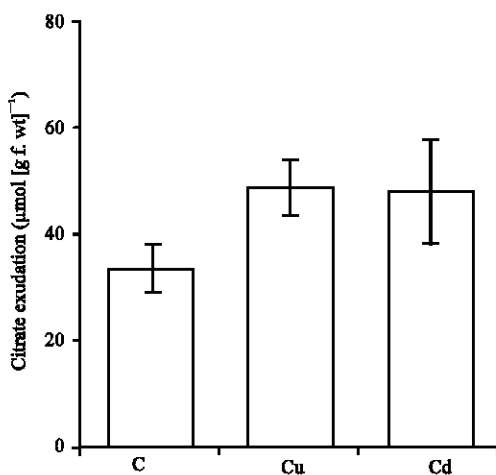


Fig. 9: Effects of copper and cadmium treatments on citrate exudation. The maize (*Zea mays* L.) seedlings were treated with 100 µM Cu or Cd for 4 days. The root exudates were collected and then analyzed by HPLC. Histograms are means±SE (n = 3) of three independent experiments

56.9%, respectively with Cu and Cd. By contrast, this activity increased 1.9 and 2.1-fold, respectively in Cu and Cd-treated shoots (Fig. 7B).

**Organic acid exudation:** Organic acid profiles of root exudates showed only one peak with Rt corresponding to citrate (Fig. 8). Both Cu and Cd did not affect the pattern of citrate exudation (Fig. 9).

## DISCUSSION

### Effects of Cu and Cd on growth and on Cu and Cd content:

The result of the current study indicated that root fresh and shoot dry wt was more affected by Cu than by Cd (Figs. 1A and D). There have been a number of studies that reported that Cu inhibited the growth even at low concentrations. Cu produced 50% growth inhibition of *Lolium perenne* root at copper concentrations not exceeded 0.02 ppm. In *Zea mays*, growth was inhibited by 0.06 ppm (Stiborova *et al.*, 1986b). In the same way, 0.6 ppm inhibited root and shoot growth in *Oryza sativa* (Gupta and Mukherji, 1977). Enhanced susceptibility to Cu observed in roots of maize plants after Cu treatment could be related to a high accumulation of Cu in the roots and/or limited translocation of the metal to the aerial part of plants (Stiborova *et al.*, 1986b). Cathala and Salsac (1975) have reported that the roots have a tendency to accumulate higher amounts of Cu than the aboveground

of plants as found in our study (Fig. 2A). Preferential accumulation of metals in roots could be a strategy for the plants to limit metal translocation, thereby avoiding metal toxicity as well for Cu (Ouzounidou *et al.*, 1992) as for Cd (Jarvis *et al.*, 1976). Many species accumulated Cu in major part in roots: *Lolium perenne* (Jarvis and Whitehead, 1981), *Mimulus guttatus* (Macnair, 1981), *Spinacia oleracea* (Baszyński *et al.*, 1982) and *Avena sativa* (Tukendorf and Baszyński, 1985). Restricted translocation of Cu from roots to shoots is earlier reported in *Agrostis stolonifera* (Wu *et al.*, 1975). The pronounced effect of Cu on shoot growth (Fig. 1D) as compared to Cd could be attributed to more susceptibility of photosynthetic apparatus to Cu. There is evidence that Cu should be excluded from the leaves because of its inhibitory effect on photosynthesis. It has been argued that Cu is a strong inhibitor of photosynthesis in algae and higher plants. Cu-induced inhibition of photosynthetic electron chain transport by acting on the oxidant sites of PSII and the reductant sites of PSI, concomitant with inhibition of photophosphorylation and dark reactions (Shioi *et al.*, 1978a,b). Furthermore, Sandmann and Böger (1980a,b) have reported that Cu<sup>+</sup> and Cu<sup>2+</sup> ions blocked photosynthetic electron transport in *Scenedesmus* and in isolated chloroplasts of spinach plants. Similarly, exposure of maize plants to Cd causes many toxic effects. In wheat, 20 µM Cd decreased to the half the fresh wt of the roots. A similar reduction of fresh wt of the shoots was observed when 50 to 70 µM Cd were applied (Trivedi and Erdei, 1992). In this study, the relative larger amount of Cd in shoots than that of Cu (Fig. 2B) may indicate higher mobility of Cd. Salt *et al.* (1995) have suggested that the translocation of Cd from roots to shoots was likely to occur via xylem and to be driven by transpiration from the leaves. This is founded from the reduced Cd accumulation in shoots after ABA-induced stomatal closure of Indian mustard. The low levels of both metals in the cell wall fraction of roots compared to the soluble fractions of these metals in treated plants (Fig. 3A and 4), suggests that root cell wall couldn't be the best site for metal accumulation.

**Role of organic acids in Cu and Cd tolerance:** We have shown that citrate exuded in response of Cu and Cd did not differ from that from roots of control plants (Fig. 8 and 9). This result suggests that external metal detoxification was not involved in Cu and Cd tolerance. Indeed, the root secretion of organic acid was reported to be metal-specific response. Previous studies have suggested that root organic acid exudation was specifically induced by Al than by other heavy metals

such as Cu and Cd, or by other environmental stresses. Indeed, the exclusion of Al by chelation with organic acid anions excreted from root apices has been suggested to be the basis of Al tolerance mechanism in many species. Thus, under Al stress, malate was released from the roots of Al-tolerant cultivars of wheat (*Triticum aestivum*) (Delhaize *et al.*, 1993); citrate from Al-tolerant cultivars of snapbean (*Phaseolus vulgaris*) (Miyasaka *et al.*, 1991); maize (*Zea mays*) (Chaffai and El Ferjani, 2005) and *Cassia tora* (Ma *et al.*, 1997c); and oxalate from buckwheat (*Fagopyrum esculentum*) (Ma *et al.*, 1997b) and taro (*Colocasia esculenta*) (Ma and Miyasaka, 1998). In contrast, phosphorus (P) deficiency, 50  $\mu$ M lanthanum ( $\text{La}^{3+}$ ), 200  $\mu$ M manganese ( $\text{Mn}^{2+}$ ), 10  $\mu$ M cadmium ( $\text{Cd}^{2+}$ ), or 50  $\mu$ M lead ( $\text{Pb}^{2+}$ ) were not able to induce the exudation of organic acids in rye (Li *et al.*, 2002). Similarly, neither P deficiency nor application of La or ytterbium (Yb) induced secretion of organic acids in *Cassia tora* or buckwheat (Ma *et al.*, 1997b,c; Zheng *et al.*, 1998). Delhaize *et al.* (1993) have also reported that La was not able to induce secretion of malate in Al-resistant cultivars of wheat. The observed difference in organic acid exudation between Cu and Cd-treated and Al-treated plants suggested a specific role for the Al-induced activation of protein channel mediating citrate efflux. Indeed, patch-clamp studies have demonstrated that Al activated an Al-dependant anion channels in intact maize root apices, particularly sensible to Al (Piñeros and Kochian, 2001). Therefore, there is evidence that a highly effective resistance mechanisms based on internal than external tolerance mechanisms appeared to operate in the case of Cu and Cd. The internal tolerance mechanism involved metal chelation or compartmentalization of the ligand-metal complex. Evidence for a potential role of metal chelating substances such as organic acids in heavy metal tolerance was particularly demonstrated in metal accumulating species. Organic acids were reported to form strong complexes with Al ions and were implicated in internal Al detoxification in Al-accumulating species. For example, in *Hydrangea*, an Al accumulator plant, the formation of an Al-citrate 1:1 complex in leaves has been proposed as an Al tolerance mechanism (Ma *et al.*, 1997a). Also in the Al hyperaccumulator *Melastoma malabathricum* Al seems to be transported in the form of citrate, while oxalate would be the storage form in leaf vacuoles (Watanabe *et al.*, 2000). Moreover, organic acids have been found to accumulate to higher concentrations in roots and leaves of metal tolerant plants (Kishinami and Widholm, 1987). We examined the organic content and found that roots accumulated high levels of citrate in roots of Cu and Cd treated plants (Table 1) and shoots

accumulated malate in Cu-treated plants and citrate in Cd-treated ones (Table 1 and 2) suggesting that these organic acids may play a role in metal detoxification. Increases of organic acid content (particularly malate and citrate) in  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  stressed plants, observed in the present study have been correlated with increased tolerance to heavy metals, such as Al, Cu, Zn, Ni... Organic acid accumulation has been correlated with metal resistance in selected Al resistant carrot cells, where increased citrate concentrations were observed (Ojima *et al.*, 1984). Mathys (1977) has reported that Zn tolerance was associated with malate accumulation. Following the Zn tolerance model proposed by Mathys (1977), Zn was chelated by malate in the cytoplasm, transported to the vacuoles and accumulated there. The accumulation of citrate and malate has been reported in Zn-tolerant grasses of *Deschampsia caespitosa* grown in the presence of Zn (Godbold *et al.*, 1984; Thuman and Rankin, 1982). In these species, the Zn tolerance was correlated with citrate levels. Similarly, in Ni accumulating plants of 17 species, Ni accumulation was correlated with high levels of citrate in their leaves (Lee *et al.*, 1978). In cultured cells of *Nicotiana plumbaginifolia*, both the Cu and Zn resistant cells accumulated higher levels of citrate and malate than the wild type cells in the presence of the metals (Kishinami and Widholm, 1987). The tolerance to Cu or Zn of *Nicotiana plumbaginifolia* cells was in part due to the ability to accumulate higher than normal levels of citrate and malate to chelate the toxic metal ions. Thus, citrate and malate content were increased 3 and 12-fold, respectively in the metal resistant cells grown in the presence of Cu or Zn compared to wild cells grown in the absence of Cu or Zn. It has been also argued that organic acids have proposed roles in the heavy metal storage and transport in plants. Strong argument is the observation that heavy metal ions would be largely present in various forms as organic acid complexes. In buckwheat, Al in both roots and leaves is found to be bound into an Al-oxalate 1:3 complex (Ma, 2000), while in leaves with high Al concentrations, an Al-citrate complex was also identified in this specie (Shen *et al.*, 2004). The marked decrease in malate content in roots with metals was correlated to the decrease in MDH activity (Fig. 7A). We have also showed that the MDH activity was significantly stimulated by both metals in shoots (Fig. 7B). This is consistent with Lee *et al.* (1976) study, where an increase in MDH activity at 0.90 and 1.35  $\mu$ M  $\text{Cd}^{2+}$  in leaf blades of soybean seedlings, 10 days after Cd treatment was observed. The salicylic acid which was only detected in shoots of Cu and Cd treated plants (Fig. 6 and Table 2) could be a part of a general stress response. The salicylic



acid is essential in regulating the growth, the development and the microorganism-plant interactions and in Systemic Acquired Resistance (SAR) in plants (Hahlbrock and Scheel, 1989). It would be interesting to extend the study to other maize cultivars in order to correlate the tolerance with organic acid changes.

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