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Bioaccumulation of Copper by *Zea mays:* Impact on Root, Shoot and Leaf Growth

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Abstract In the present study, the growth and the Cu^{2+}accumulation by roots, shoots and leaves of *Zea mays* were examined using copper sulphate in the range of 10^{-4} to 10^{-2} M. Plants of *Z. mays* did not show inhibition of growth in the presence of 10^{-4} to 10^{-2} M Cu^{2+}; however, it was observed growth effects on root when different Cu^{2+} solution concentrations were used. Only the seedlings exposed to 10^{-2} M exhibited substantial root growth reduction, yielding only 56% of length with respect to the control. Seedlings exposed to 10^{-4} M Cu^{2+} exhibited 16% and 42% growth increase in shoots and leaves, respectively, when compared with the controls. The seedlings treated with 10^{-3} and 10^{-2} M Cu^{2+} were inhibited in shoot and leaf growth. The fresh weights in roots, shoots and leaves significantly decreased at 10^{-2} M Cu^{2+}. The tolerance index, based on root length, was not significantly different for the three different treatments with copper. However, the total accumulation rate was very low at 10^{-4} and 10^{-3} M compared to 10^{-2} Cu treatments. The capacity of copper accumulation by roots, shoots and leaves of *Z. mays* plants increased concomitant to the copper concentration, arriving to 382 times more in roots, 157 in shoots and only 16 in leaves, all compared to the controls. Cu could be accumulated by roots, shoots and leaves when the initial concentrations were 10^{-3} and 10^{-4} M. However, when it was 10^{-2} M, the metal could not be accumulated by leaf and shoot levels; the roots could increase their copper accumulation capacity three times compared to the control. *Z. mays* has potential ability to accumulate Cu without being overly sensitive to Cu toxicity.

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1 Introduction

Metals are natural components in soil with a number of heavy metals being required by plants as micronutrients. However, pollution of biosphere by toxic metals has accelerated dramatically since the beginning of the industrial revolution.

Cu is one of common metal contaminants in many parts of the world. Cu contamination usually results from human activities, such as mining, smelting, industrial waste disposal, sewage sludge application to agricultural soils and the use of fertilizer and pesticide (Wei et al. 2008).

Cu is an essential element for plants, being associated with a large number of enzymes, which catalyse oxidative reactions in a variety of metabolic pathways (Marschner 1995). Plants require approximately 5–30 mg Cu kg^{-1} dry weight (DW) for normal growth (Kabata-Pendias and Pendias 1992); Cu deficiency usually occurs when plant Cu concentration is smaller than 5 mg kg^{-1} dry weight (Marschner

1995). When it is absorbed in excess amount, Cu can be considered as a toxic element, leading to total inhibition of growth, with disturbance of the mitosis, inhibition of root elongation and damage to root epidermal cells and root cell membranes (Ouzounidou et al. 1995). Copper is also thought to affect a wide range of biochemical and physiological processes, such as photosynthesis, pigment synthesis, nitrogen and protein metabolism, membrane integrity and mineral uptake (Luna et al. 1994; Shen et al. 1998; Nielsen et al. 2003; Demirevska-Kepova et al. 2004). According to Kabata-Pendias and Pendias (1984), 60 to 125 mg/kg Cu, based on total fractions in soil, would be considered toxic to plants. Excessive Cu may be toxic not only to plants but also to human beings via the food chain and may thus pose a potential threat to human health. Remediation strategies are therefore needed for Cu-contaminated soils (Weng et al. 2005).

Emphasis has become more prevalent towards the problems of Cu pollution with the development of modern industry and agriculture. While soil cleanup techniques, such as isolation and containment, mechanical separation, chemical treatment or soil flushing, have proven to be effective in small areas, they require special equipment and intensive labour (Mulligan et al. 2001). Furthermore, these methodologies are not only costly but they also cause soil disturbances and are not readily accepted by communities. Phytoremediation, the use of plants to restore polluted sites, has recently become a tangible alternative to traditional methodologies (Glass 2000; Lasat 2002; Jing et al. 2007). It has been established that certain wild and crop plant species have the ability to accumulate elevated amounts of toxic heavy metals (Reeves and Baker 2000; Ghosh and Spingh 2005; Brunet et al. 2008). Thus, researchers all over the world are searching new plant species susceptible to be used in heavy metals phytoremediation (Rai et al. 2002; Del Rio et al. 2002; Wang et al. 2007).

Maize (*Zea mays*) is one of the most important cereal crops. However, few reports on copper accumulation by maize are available. Liu et al. (2001) studied the uptake and accumulation by roots and shoots of maize. They found that root growth decreased progressively with increased concentrations of Cu^{2+} in solution, but the shoot growth was similar to the control. However, the plants transported and concentrated only a small amount of copper in their roots.

Based on such information, the aim of this investigation was to study the effects of different Cu^{2+} concentrations on the root, shoot and leaf growth of maize (*Z. mays*) of Tucumán, Argentina, and the copper uptake and accumulation by the plants.

2 Materials and Methods

2.1 Chemicals and Seeds

All chemicals used in the cultivation medium and reagents were of analytical grade and were purchased from standard companies. Seeds of maize (*Z. mays* L. Cargill 350 Hybrid) healthy and equal-sized were chosen.

2.2 Seed Germination

Selected seeds of maize were soaked in water in a container (24 h). The seed germination was carried out on moist filter paper. Seeds were placed on Whatman no. 1 filter paper discs (at 9.0 cm, sterile) kept in Petri dishes (10.0 cm diameter) and moistened with destilled water. In each Petri dish, 12 seeds were germinated at ambient temperature (28–30°C) under darkness. To maintain moisture, 1 ml of sterile distilled water was added every alternate day.

Following germination, seedlings were transplanted and grown in glass cups (8.0 cm diameter) filled with 50 g vermiculite. The cups were kept at ambient temperature (28–30°C) in a germinator (14-h photoperiod), and 10.0 ml sterile modified Hoagland's nutrient

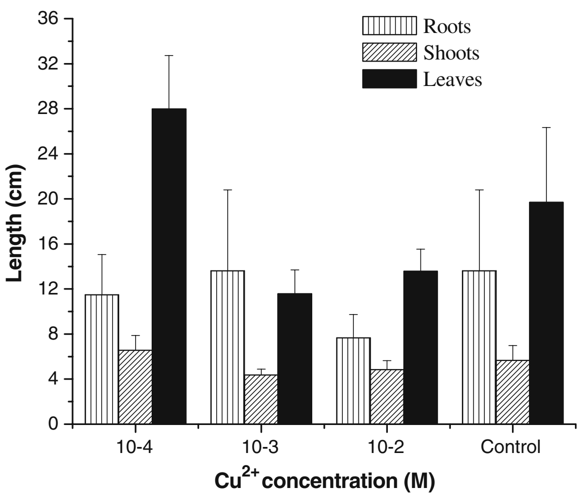

Fig. 1 Effect of different concentrations of Cu^{2+} on roots, shoots and leaves growth of *Z. mays*. *Vertical bars* denote standard error (n=15)

Table 1 Effects of Cu^{2+} on fresh weight of roots, shoots and leaves of *Z. mays*

Treatment (M)[a]	Roots (g ± SE)	Shoots (g ± SE)	Leaves (g ± SE)
Control	0.50±0.10	0.21±0.10	0.48±0.20
10^{-4}	0.51±0.10	0.25±0.06	0.66±0.20
10^{-3}	0.60±0.10	0.14±0.03	0.25±0.10
10^{-2}	0.35±0.08	0.18±0.06	0.29±0.10

Means ± SE, $n=15$

SE standard error

[a] M = moles per litre

solution was added to each cup every day to maintain moisture. The modified Hoagland's nutrient solution (Stephan and Prochazka 1989) contains the following nutrients: 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 1 mM KH_2PO_4, 5 μM H_3BO_3, 1 mM $MgSO_4$, 4.5 μM $MnCl_2$, 3.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, 0.1 mM $(NH_4)_6Mo_7O_{24}$ and 10 μM ferric ethylenediaminetetra-acetic acid.

After a 10-day culture, the seedlings were watered with nutrient solution supplemented with different concentrations of Cu (10^{-2}, 10^{-3} and 10^{-4} M Cu^{2+}). Copper was provided as copper sulphate ($CuSO_4.5H_2O$). The solutions were prepared with deionised water. Seedlings watered with the Hoagland's nutrient solution were used for the control.

Ten seedlings from each treatment were harvested based on uniformity in size and colour after 6 days of Cu^{2+} treatment, and their roots were rinsed in deionised water to remove traces of nutrient and Cu^{2+} ions on the surfaces of the roots. Fresh weight was measured after the seedlings were divided into roots, shoots and leaves. The samples were dried for 3 days at 40°C, dried again for 12 h at 105°C in oven, measured for DW and then ashed for 2 h at 200°C and 10 h at 600°C.

2.3 Analytical Procedures

The element Cu was determined by atomic absorption spectrometry (AAnalyst 100, Perkin Elmer) after dry ashing, as described by Hou (1991). The tolerance index (TI) and accumulation rate of *Z. mays* were calculated by equations described by Shu et al. (2002) as follows:

$$\text{tolerance index}(\%) = \frac{\text{mean length of longest root in presence of added Cu}}{\text{mean length of longest root in unamended control}} \times 100$$

$$\text{accumulation rate}(\text{mg/g} \times \text{DW/day}) = \frac{([\text{metal}]\text{root} \times \text{DWroot} + [\text{metal}]\text{shoot} \times \text{DWshoot} + [\text{metal}]\text{leave} \times \text{DWleave})}{6 \times (\text{DWroot} + \text{DWshoot} + \text{DWleave})}$$

2.4 Statistical Analyses

Statistical analyses were conducted using the Microcal™ OriginWorking Model Version 6.0. Paired *t* test and variance analysis were used with a probability level of $p<0.05$.

Table 2 TI of roots and total metal accumulation rate expressed as micrograms per gram DW per day

Treatment (M)[a]	TI (%)	Accumulation rate (μg g^{-1} DW day^{-1})
10^{-4}	82.6	2.71
10^{-3}	91.3	4.00
10^{-2}	82.6	156.41

DW dry weight, *TI* tolerance index

[a] M = moles per litre

3 Results

3.1 Macroscopic Effects of Cu^{2+} on Root, Shoot and Leaf Growth

Plants from all populations of *Z. mays* grew well in the presence of 10^{-4} to 10^{-2} M Cu^{2+} with a similar leaf colour to those grown under control conditions; however, the effects of Cu^{2+} on roots growth of *Z. mays* varied with different concentrations of copper sulphate solutions used (Fig. 1). Neither the 10^{-4}- nor 10^{-3} M Cu^{2+} treatments caused significant

Table 3 Copper accumulation by roots, shoots and leaves of *Z. mays* after 6 days treatment

Treatment (M)[a]	Roots (μg/g DW ± SE)	Shoots (μg/g DW ± SE)	Leaves (μg/g DW ± SE)
Control	4.37±1.70	3.78±2.68	10.21±5.76
10^{-4}	5.92±0.70	5.83±1.68	13.57±1.76
10^{-3}	8.34±0.10	6.52±0.16	22.06±3.49
10^{-2}	1,668.25±23.28	594.82±2.73	160.97±31.71

DW dry weight, *SE* standard error

[a] M = moles per litre

changes in roots length compared to the control roots. Seedlings exposed to 10^{-2} M Cu^{2+} solution exhibited substantial root growth reduction, yielding only 56% of the root length of the control. They appeared thinner and the root tips were slightly blue.

The effect of Cu^{2+} on shoot and leaf lengths varied depending on concentration (Fig. 1). Seedlings exposed to 10^{-4} M Cu^{2+} solution exhibited growth increase in shoots and leaves, increasing 16% and 42%, respectively, when compared with the shoot and leaf lengths of the control seedlings. The seedlings treated with 10^{-3} and 10^{-2} M Cu^{2+} were inhibited in shoot and leaf growth; they were smaller and appear slightly yellow.

Cu^{2+} can, to some degree, cause partial improvement of fresh weight of the roots, shoots and leaves of *Z. mays* (Table 1). The fresh weights in roots, shoots and leaves slightly increased or decreased in the groups treated with 10^{-4} and 10^{-3} M Cu^{2+}. At 10^{-2} M Cu^{2+}, they significantly decreased. These phenomena indicated that the plant growth is sensitive to higher concentration of Cu^{2+} (10^{-2} M).

The TI, based on root length, was not significantly different for the three copper treatments, indicating that the sensitivity of the plant was similar in all the studied cases. However, the total accumulation rate of Cu was very low (almost ten times less) at 10^{-4} and 10^{-3} M Cu^{2+} treatments compared to 10^{-2} (Table 2).

3.2 Cu Accumulation

Accumulation of Cu in roots, shoots and leaves of *Z. mays* considerably varied, depending on different Cu concentration used (Table 3). It is important to notice that the capacity of copper accumulation increased concomitant to the copper concentration, arriving to 382 times more in roots, followed by 157 in shoot and only 16 in leaves, all compared to the respective controls.

When the copper accumulation average is compared in relation to the initial concentration added to the plant (Table 4), it is important to show that this metal could be accumulated by roots, shoots and leaves when the initial concentrations were 10^{-3} and 10^{-4} M; these results were similar to the controls. However, when the Cu^{2+} concentration was 10^{-2} M, the metal could not be accumulated by leaves, also at the shoots level, and the roots could increase their copper accumulation capacity three times compared to the control, probably due to the interference of the high Cu concentration in the nutrient solution.

4 Discussion

Excess Cu can produce toxic effects on plants, such as inhibiting plant growth, causing chlorosis of leaves, increasing root cell membranes leakage (Shen et al. 1998; Murphy et al. 1999). Ait Ali et al. (2002) found that root length of reed and maize seedlings was more sensitive than other measured growth parameters. The results found in the present study showed that seedlings treated with 10^{-3} and 10^{-2} M Cu^{2+} were inhibited in shoot and leaf growth but not in root (Fig. 1). Meng et al. (2007) studied the effect of 10^{-4} M and 10^{-3} M Cu^{2+} on the garlic seedlings founding a significant growth reduction of these seedlings.

Table 4 Distribution changes of copper ion amounts in roots, shoots and leaves of *Z. mays* after treatment with different concentrations of Cu

Treatment (M)[a]	Total amount (μg/g DW)	Roots (%)	Shoots (%)	Leaves (%)
Control	18.36	23.8	20.6	55.6
10^{-4}	25.32	23.4	23	53.6
10^{-3}	36.92	22.6	17.6	59.8
10^{-2}	2,424.04	68.8	24.5	6.7

DW dry weight

[a] M = moles per litre

The root had similar TI at the three Cu concentrations studied; however, the total metal accumulation rate by the seedlings was increased more than 30 times at 10^{-2} M Cu^{2+}. These results agree with the capacity of copper accumulation when the Cu concentration was increased, mainly in roots and shoots (Table 1). These observations indicate that maize plants can tolerate and accumulate high Cu concentrations without visible morphological changes.

Even, after 6 days of treatment, the seedling increased the capacity of copper accumulation in roots, shoots and leaves (68.8%, 24.5% and 6.7%, respectively) when 10^{-2} M Cu^{2+} was added with the nutrient solution (Table 4). Liu et al. (2001) found that the Cu content in roots of *Z. mays* increased with increased solution concentration of Cu^{2+}; however, they could not find significant Cu accumulation in shoots, and also they did not studied this behaviour in leaves.

Cu^{2+} is required by biological systems as a structural and catalytic enzyme component, and in the soil, Cu^{2+} can be a stress factor by causing physiological responses that can decrease the vigour of the plants and inhibit plant growth (Ouzounidou 1994). Cu^{2+} pollution has become a major environmental problem due to the long term use of copper-containing fungicides, industrial and urban activities (e.g. air pollution, city waste and sewage sludge) and the application of pig and poultry slurries high in copper (Marschner 1995).

Phytoremediation has been also considered as an emerging technology using selected and engineered metal-accumulating plants for environmental cleanup. Many studies on uptake and accumulation of heavy metals by plants have been reported recently. Wei et al. (2008) found that concentrations of Cu accumulated in plants of *Chrysanthemum coronarium* L. and *Sorghum sudanense* L. increased greatly with the increasing Cu level in the treatments. Ucun et al. (2009) proposed the use of *Pinus sylvestris* L. biomass as biosorbent for removing Zn(II) and Cu (II), and the maximum biosorption efficiency was 67% for Cu(II).

Maize (*Z. mays* L.) can be chosen as plant species for phytoremediation because of its high biomass yields and heavy metal tolerance. Ait Ali et al. studied maize tolerance and proposed this plant as a possible solution for the stabilisation and restoration of Cu-polluted soils. Additionally, maize may create particularly good environmental conditions for soil microorganisms and microfauna (Lin et al. 2008).

However, few reports on copper accumulation by wild *Z. mays* from Argentina are available. The results from this investigation indicated that *Z. mays* plants have the potential ability to remove and accumulate Cu^{2+} from aqueous solutions. Yet, to our knowledge, no study has demonstrated copper accumulation in maize grain, even in Argentina.

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