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UPTAKE AND ACCUMULATION OF CADMIUM AND SOME NUTRIENT IONS BY ROOTS AND SHOOTS OF MAIZE (*ZEA MAYS* L.)

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Abstract

The effects of the different concentrations of Cd on accumulation of four cultivars of maize (*Zea mays* L.) viz., Nongda No. 108, Liyu No. 6, Shendan No. 10 and Tangkang No. 5, were investigated using inductively coupled plasma atomic emission spectrometry (ICP-AES). The Cd accumulation in the roots and shoots and the interactions among Mn, Fe and Cu were also analyzed in the present study. The concentrations of cadmium chloride ($CdCl_2 \cdot 2.5\ H_2O$) used were in the range of 10^{-4} M to 10^{-6} M. The results indicated that the Cd levels in roots and shoots of four cultivars increased significantly ($P < 0.005$) with increasing Cd concentration. Cadmium ions were concentrated mainly in the roots, and small amounts of Cd were transferred to the shoots. In Liyu No. 6, the distributive levels of Cd in the roots decreased with increasing the concentration of Cd. Liyu No. 6 had more ability to remove Cd from solutions and accumulate it when compared with the other cultivars. This kind of cultivar with many roots, a high biomass and high ability to accumulate Cd can play a very important role in the soil contaminated by Cd.

Introduction

The most common heavy metals in the environment are Cd, Cr, Cu, Hg, Pb and Zn. Recently, the industrial and agricultural development has released enormous amount of heavy metals and polluted the environment. Cadmium is particularly a dangerous pollutant due to its high toxicity and great solubility in water. At higher concentrations, it characteristically inhibits growth of different plant species such as maize (Lagriffoul *et al*., 1998), barley and wheat (Talanova *et al*., 2001), and garlic (Liu *et al*., 2003/2004). It also induces leaf chlorosis accompanied by a lowering of photosynthetic rate (Bazzaz *et al*., 1974; Hampp *et al*., 1976; Bazynski *et al*., 1980; Das *et al*., 1997), disturbs cell proliferation (Rosas *et al*., 1984), impedes respiration (Lee *et al*., 1976), reduces mitochondrial electron transport (Miller *et al*., 1973), induces high vacuolization in cytoplasm and nucleoli, and increases disintegration of organelles (Liu & Kottke, 2003). Considerable importance has been attached to the problems associated with Cd pollution. However, most conventional remediation approaches do not provide acceptable solutions to toxic metal pollution. Phytoremediation is an emerging technology that employs the use of higher plants for the cleanup of contaminated environments (Lasat, 2000).

Maize (*Zea mays* L.) is one of the most important cereal crops and comprises some heavy metal tolerant genotypes (Clark, 1977; Liu *et al*., 2001). It is very important for scientists to find some maize cultivars with the capability of absorbing and accumulating extraordinarily high amounts of heavy metals from soil. Although the effects of Cd on maize were studied by several authors (Lagriffoul *et al*., 1998; Ju *et al*., 1997), a few

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investigations concerning Cd accumulation and its effects on other heavy metals have been reported. In order to select a suitable maize cultivar with high uptake and accumulation of Cd^{2+} without undergoing severe damage to plant and to obtain more information about the mechanism of detoxification or tolerance of Cd by interacting with other metals, this study was conducted. The uptake and accumulation of Cd by roots and shoots of maize and effects of Cd on Fe, Mn and Cu were investigated in this study using inductively coupled plasma atomic emission spectrometry (ICP-AES).

Materials and Method

Seeds of four cultivars of maize (*Zea mays* L.) *i.e.* Nongda No. 108, Liyu No. 6, Shendan No. 10 and Tangkang No. 5, were provided by the Institute of Crops, Tianjin Academy of Agricultural Sciences, Tianjin, P. R. China for use in the present investigation. Healthy and equal-sized seeds were chosen from each variety, soaked in tap water for 24 h, and germinated in the dark (25 ºC). Following germination, 20 seedlings for each treated group were chosen, fixed in cystose, and were floated on the 1/2 modified Hoagland's nutrient solution (Stephan & Prochazka, 1989) in plastic containers in a greenhouse equipped with supplementary lighting (14-h photoperiod; 24-26 °C). The container contained 2L Hoagland's nutrient solution consisted of 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 1 mM KH_2PO_4, 50μM H_3BO_3, 1 mM $MgSO_4$, 4.5 μM $MnCl_2$, 3.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$ and 0.1 mM $(NH_4)_6Mo_7O_{24}$ and 10 μM FeEDTA at pH of 5.5. Three days after culture, the seedlings were treated with Cd. Four concentrations of Cd (0, 10^{-6} M, 10^{-5} M and 10^{-4} M) were added to each container having 2 L aerated Hoagland's nutrient solution. Cadmium was provided as cadmium chloride ($CdCl_2 \cdot 2.5\ H_2O$). The Cd solutions were prepared in deionized water. The test solutions were changed every 4 days. Ten plantlets from each treatment were harvested randomly based on uniformity of size and color after 20 days of incubation. Their roots were rinsed in deionized water to remove traces of nutrient and Cd ions on the surface, and detached from shoots. The samples were dried for 3 days at 45 ºC, followed by 3 days at 80 ºC in oven, measured for dry weight (DW), and ashed for 2 h at 200 ºC and then placed for 10 h at 650 ºC. The contents of Cd, Mn, Cu and Fe were determined with inductively coupled plasma atomic emission spectrometry (ICP-AES) (LEEMAN LABS INC., New Hampshire, USA) after dry-ashing, as described by Duan (2003).

Analysis of variance (ANOVA) using sigma statistical software (Jandel Scientific Corporation) was performed. Test of equality of averages using *t*-test was applied equally. The statistical significance was set at the $P < 0.005$ confidence level.

Results

Cd uptake and accumulation: The Cd uptake and accumulation in roots and shoots of four maize cultivars varied depending on the different Cd concentrations used. As shown in Table 1, the Cd content in roots and shoots increased significantly ($p < 0.005$) with increasing Cd concentration. Cadmium ions were accumulated mainly in the roots, and small amounts of Cd were transferred to the shoots. In Liyu No.6, the distributive levels of Cd in roots decreased with the increasing concentrations of Cd. For other 3 cultivars, the distributive levels of Cd in roots exposed to 10^{-4} M Cd were the lowest when compared to those in 10^{-5} and 10^{-6} M Cd solutions. Liyu No. 6 proved more capable to remove and accumulate Cd from solutions as compared with other 3 cultivars.

Table 1. Cadmium uptake by four cultivars of *Zea mays* L.

Cultivars	Treatments (Cd M)	Metal amount (µg/g)				
		Root (DW ± SE)	Shoot (DW ± SE)	Total amount (DW)	Root (%)	Shoot (%)
Nongda No.108	Control	15.6 ± 0.09 a	1.9 ± 0.02 a	17.5	89.1	10.9
	10^{-6}	235.8 ± 0.89 b	105.6 ± 0.23 b	341.4	69.1	30.9
	10^{-5}	527.4 ± 3.60 c	172.1 ± 0.87 c	699.5	75.4	24.6
	10^{-4}	634.8 ± 1.70 d	467.1 ± 6.40 d	1101.9	57.6	42.4
Liyu No. 6	Control	36.1 ± 0.12 a	6.7 ± 0.01 a	42.8	84.3	15.7
	10^{-6}	465.1 ± 0.95 b	103.4 ± 1.34 b	568.5	81.8	18.2
	10^{-5}	1021.4 ± 6.51 c	227.2 ± 0.58 c	1248.6	81.8	18.2
	10^{-4}	1288.4 ± 13.31 d	600.1 ± 0.73 d	1888.5	68.2	31.8
Shendan No. 10	Control	59.0 ± 1.83 a	7.8 ± 0.10 a	66.8	88.3	11.7
	10^{-6}	287.8 ± 0.59 b	105.3 ± 0.30 b	393.1	73.2	26.8
	10^{-5}	651.2 ± 1.18 c	199.1 ± 3.97 c	850.3	76.6	23.4
	10^{-4}	633.8 ± 0.91 d	414.4 ± 2.96 d	1048.2	60.5	39.5
Tangkang No. 5	Control	44.2 ± 0.23 a	12.2 ± 0.03 a	56.4	78.4	21.6
	10^{-6}	409.2 ± 1.73 b	106.3 ± 0.33 b	515.5	79.4	20.6
	10^{-5}	773.1 ± 2.01 c	159.4 ± 5.92 c	932.5	82.9	17.1
	10^{-4}	913.5 ± 5.61 d	360.9 ± 3.85 d	1274.4	71.7	28.3

Means followed by corresponding SE bars and letters indicate significant differences among means. Values followed by same letters are not significantly different at ($P > 0.005$). Means ± SE, n= 3

The effects of Cd on Mn, Fe and Cu uptake and accumulation

The effects of Cd on Fe, Mn and Cu concentration in maize cultivars varied with the concentrations of Cd added. Mn uptake and accumulation significantly ($P < 0.005$) decreased with increasing Cd ions in nutrient solution in all maize cultivars (Table 2). The distributive levels of Mn in roots also decreased with increasing Cd concentrations except for Liyu No.6 treated with 10^{-6} M Cd. Mn content in the control was the highest except for the one of Liyu No. 6 exposed to 10^{-6} M Cd (Table 3).

Seedlings of the 4 cultivars could uptake and accumulate Fe significantly ($P < 0.005$) after the treatments with different concentration of Cd (Table 2). The results showed that Fe levels of the treated groups, Nongda No. 108 and Tangkang No. 5, were higher than their controls (Table 2). However, the Fe content in Shendan No. 10 treated with Cd was lower than its control (Table 2). In all cultivars Fe ions were mainly accumulated in the roots and only small amounts of Fe were transferred to the shoots (Table 3).

Cu content in roots of Nongda No. 108 increased with the increasing concentrations of Cd, while it decreased in the roots of Liyu No. 6 and Tangkang No. 5. The contents of Cu in roots of Nongda No. 108 and Shendan No. 10 treated with Cd were higher than those in controls, and Tangkang No. 5 and Liyu No. 6 lower (Table 2). In all the cultivars more than 80 % of Cu was accumulated in the roots (Table 3). The effects of Cd on Cu content in shoot of four cultivars were not significant.

Discussion

Efficiency of phytoextraction is relative to the ability of the plant to grow on polluted soils and produce a large biomass with high concentration of metal in the above-ground parts (Schwartz *et al.*, 2001). There are many reports on the definition of hyperaccumulation (Baker & Brook, 1989; Baker *et al*., 2000; Köhl *et al.*, 1997). Most recognized standard criteria base on metal amounts in above-ground tissues on a dry-biomass basis of plant material sampled from the natural habitat (Pollard *et al.*, 2002). According to the current accepted hyper-accumulation definition shoot concentration being 0.01% (on a w/w basis) for cadmium (Baker *et al*., 2000), the four cultivars appear to be Cd-hyper-accumulator, because the Cd contents in the shoots reach or exceed the standard criteria. However, only Liyu No. 6 can be considered as Cd-hyperaccumulator, because it grew very strong under concentrations of 10^{-5} M and 10^{-6} M Cd, and it was inhibited at concentration of 10^{-4} M Cd. This kind of cultivar with many roots, a high biomass and high ability to accumulate Cd can play a very important role in the soil contaminated by Cd. The results in the present investigation also demonstrated that Liyu No. 6 has obvious ability to uptake and accumulate Cd ions as compared to other three cultivars. Cadmium ions mainly accumulated in the roots with lower concentrations in shoots, which agree with the findings of Lagriffoul *et al*., (1998). These differences in root and shoot uptake might be explained by the fact that one of the normal functions of roots is to selectively acquire ions from the soil solution, whereas shoot tissue does not normally play this role (Salt *et al.*, 1997). The accumulation of Cd decreased from epidermis to inner parts of the root cortex. As the endodermis constitutes a barrier to ion transport, root cortex cells usually contain higher element concentrations than cells in the central vascular cylinder (Hagemeyer & Breckle, 1996).

Table 2. Accumulation of Mn, Fe and Cu in four maize cultivars exposed to different concentrations of Cd.

Cultivars	Treatments (Cd M)	Mn (µg/g DW ± SE)		Fe (µg/g DW ± SE)		Cu (µg/g DW ± SE)	
		Root	Shoot	Root	Shoot	Root	Shoot
Nongda No.108	Control	340.8 ± 1.92 a	88.7 ± 0.39 a	253.1 ± 0.47 a	56.4 ± 0.52 a	54.5 ± 0.26 a	13.4 ± 0.12 a
	10^{-6}	40.9 ± 0.10 b	61.2 ± 0.10 b	418.6 ± 0.47 b	96.1 ± 0.55 b	68.8 ± 0.42 b	13.0 ± 0.12 a
	10^{-5}	17.9 ± 0.08 c	78.0 ± 2.51 c	540.2 ± 4.07 c	81.1 ± 0.99 c	103.8 ± 1.29 c	12.4 ± 0.19 a
	10^{-4}	8.5 ± 0.55 d	37.5 ± 0.15 d	584.0 ± 1.36 d	82.0 ± 1.12 c	127.0 ± 0.60 d	13.4 ± 0.03 b
Shendan No.10	Control	264.6 ± 0.51 a	80.0 ± 0.17 a	476.1 ± 0.75 a	85.8 ± 0.07 a	72.4±0.18 a	18.9±0.09 a
	10^{-6}	178.8 ± 0.78 b	98.7 ± 0.22 b	254.8 ± 0.89 b	94.6 ± 0.30 b	150.8 ± 1.07 b	13.0 ± 0.09 b
	10^{-5}	27.9 ± 0.24 c	77.8 ± 2.01 c	383.2 ± 5.25 c	88.9 ± 0.39 b	67.0 ± 0.42 c	9.8 ± 0.08 c
	10^{-4}	9.9 ± 0.28 d	30.3 ± 0.29 d	406.4 ± 5.96 c	87.9 ± 0.58 b	360.4 ± 10.25 d	27.4 ± 0.12 d
Liyu No.6	Control	80.7 ± 0.68 a	67.3 ± 0.18 a	564.3 ± 1.52 a	77.1 ± 0.28 a	124.9±0.55 a	27.8±0.10 a
	10^{-6}	212.3 ± 0.09 b	147.2 ± 6.66 b	389.1 ± 1.67 b	75.0 ± 2.44 a	104.7 ± 0.90 b	13.5 ± 0.11 b
	10^{-5}	69.7 ± 0.37 c	66.8 ± 0.06 c	420.8 ± 2.19 c	61.6 ±0.10 b	83.6 ± 0.50 c	10.2 ± 0.03 c
	10^{-4}	17.1 ± 0.03 d	53.9 ± 0.61d	824.5 ± 3.26 d	58.3 ± 0.33 c	49.5 ± 0.72 d	11.2 ± 0.13 c
Tangkang No.5	Control	160.1 ± 0.72 a	63.9 ± 0.27 a	413.3 ± 0.44 a	70.2 ± 0.15 a	276.3 ± 0.89 a	22.5 ± 0.15 a
	10^{-6}	125.3 ± 0.49 b	43.7 ± 0.25 b	500.9 ± 0.96 c	64.8 ± 0.15 b	134.5 ± 1.93 b	9.7 ± 0.10 b
	10^{-5}	21.1 ± 0.10 c	54.4 ± 1.60 c	566.7 ± 3.10 c	63.8 ± 0.83 b	60.9 ± 0.59 c	9.8 ± 0.04 b
	10^{-4}	10.5 ± 0.03 d	28.1 ± 0.10 d	491.3 ± 2.43 d	76.4 ± 0.34 c	43.5 ± 0.48 d	10.8 ± 0.19 c

Means followed by corresponding SE bars and letters indicate significant differences among means. Values followed by same letters are not significantly different at ($P > 0.005$). Means ± SE, n= 3

Table 3. Distributive changes of Mn, Fe and Cu ions in root and shoot of four maize cultivars treated with different concentrations of Cd.

Cultivars	Treatments (Cd M)	Mn			Fe			Cu		
		Total amount (µg/g DW)	Root (%)	Shoot (%)	Total amount (µg/g DW)	Root (%)	Shoot (%)	Total amount (µg/g DW)	Root (%)	Shoot (%)
Nongda No.108	Control	429.5	79.3	20.7	309.5	81.8	18.2	67.9	80.3	19.7
	10^{-6}	102.1	40.1	59.9	514.7	81.3	18.7	81.8	84.1	15.9
	10^{-5}	95.9	18.7	81.3	621.3	86.9	13.1	116.2	89.3	10.7
	10^{-4}	46.0	18.5	81.5	666.0	87.7	12.3	140.4	90.5	9.5
Shendan No.10	Control	344.6	76.8	23.2	561.9	84.7	15.3	91.3	79.3	20.7
	10^{-6}	277.5	64.4	35.6	349.4	72.9	27.1	163.8	92.1	7.9
	10^{-5}	105.7	26.4	73.6	472.1	81.2	18.8	76.8	87.2	12.8
	10^{-4}	40.2	24.6	75.4	494.3	82.2	17.8	387.8	92.9	7.1
Liyu No.6	Control	148.0	54.5	45.5	641.4	88.0	12.0	152.7	81.8	18.2
	10^{-6}	359.5	59.1	40.9	464.1	83.8	16.2	118.2	88.6	11.4
	10^{-5}	136.5	51.1	48.9	482.4	87.2	12.8	93.8	89.1	10.9
	10^{-4}	71.0	24.1	75.9	882.8	93.4	6.6	60.7	81.5	18.5
Tangkang No.5	Control	224.0	71.5	28.5	483.5	85.5	14.5	298.8	92.5	7.5
	10^{-6}	169.0	74.1	25.9	565.7	88.5	11.5	144.2	93.3	6.7
	10^{-5}	75.5	27.9	72.1	630.5	89.9	10.1	70.7	86.1	13.9
	10^{-4}	38.6	27.2	72.8	567.7	86.5	13.5	54.3	80.1	19.9

Hagemeyer & Breckle (1996) reported that the contents (mg kg^{-1} DW) of several essential trace metals are quite different in leaves of higher plants. For instance, Fe: 60-1500, Mn: 20-900, Cu: 2-20. Data from the present investigation fall in these.

Manganese (Mn) is an essential element required in trace amounts by all organisms. It plays a crucial role in the life-cycle of plants and other photosynthetic organisms, because it is required for light-induced evolution of oxygen from water (Andersson & Styring, 1991). A number of metabolic and protein-processing enzymes require Mn as a cofactor (Kaufman *et al.*, 1994; Larson & Pecoraro, 1992). Larson & Pecoraro (1992) also reported it as a redox-active cofactor in Mn-superoxide dismutase and its important role in the detoxification of free radical forms of oxygen. Korshunova1 *et al*., (1999) demonstrated that the IRT1 protein which had previously been identified as an iron transporter when expressed in yeast could transport manganese as well, but the manganese uptake activity was inhibited by Cd^{2+}, Fe^{2+} and Zn^{2+}. The results from this investigation showed that the uptake and accumulation of Mn were reduced significantly ($P < 0.005$) in the cultivars treated with Cd, which agrees with the findings by Hernández *et al*., (1996, 1998).

Iron is an essential element for plant growth because it is required in the activities of a range of enzymes, especially those involved in oxidation and reduction processes, for the synthesis of porphyrine ring (chlorophyll and heme biosynthesis), reduction of nitrite and sulphate, N_2-fixation (as a part of the leghemoglobin), etc. (Rengel, 1999). It is known that the lack of iron in growth media leads to the accumulation of Cd, Cu, Mn and Zn in shoots and roots of pea (Cohen *et al.*, 1998). Hagemeyer & Breckle (1996) reported that Fe plays a general role in cation absorption of roots, the enzyme catalyzes the reduction of Fe^{3+} to Fe^{2+}, which is more readily absorbed by roots, the plasma membrane-bound reductase system can also increase the uptake of other trace elements, like copper and manganese. Fe uptake in the Cd treated-plants was greater than that in control plants in Nongda No.108 and Tangkang No.5, which is in agreement with the findings by Hernández *et al*., (1998), who found that Fe in pea plants was higher than that recorded in the control plants after the treatment with 50 µM Cd. The relationship between Fe and Cd uptake is significant, as reported by Lombi's *et al*., (2002): both short and long-term studies revealed that Cd uptake was significantly enhanced by Fe deficiency in the Ganges ecotype.

Within a certain concentration range, copper was extensively translocated, as it was essential to the plant metalloenzymes diamine oxidase, ascorbate oxidase, cytochrome C oxidase, superoxide dismutase and plastocyanin oxidase (Van Assche & Clijsters, 1990) and photosynthesis (Hsu & Lee, 1988). Various interactions can occur when plants are exposed to unfavorable concentrations of more than one trace element. The sensitive plants showed chlorosis and necrosis of young leaves in response to Cu stress. The results from this investigation indicated that the plants concentrated the Cu^{2+} in the roots more than that of the above-ground parts of maize cultivars when treated with different concentrations of Cd, which disagrees with the finding of Liu *et al*. (2001) who reported a large amount of Cu accumulation in the shoots (10^{-4} M and 10^{-5} M Cd) of *Zea mays*.

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