The Effect of EDTA on Maize Seedlings Response to Cd-Induced Stress

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Growth parameters of maize seedlings were studied as well as cadmium content in plants in function of the EDTA dose applied to the nutrient medium. No increase in Cd accumulation was found in plants under the influence of EDTA, but this chelating agent was observed to suppress the effect of Cd toxicity on the plant growth.

Introduction

The possibility of utilizing plants for soil decontamination of heavy metals received more and more interest in recent years. Plants useful for phytoremediation should be characterized by a high biomass production and by accumulation of high amounts of metals in easily harvested shoots (Blaylock *et al.,* 1997). These conditions are fulfilled by some cultivars of Indian mustard (*Brassica juncea*), but other agronomic crops, such as maize, in combination with soil amendments, can also be useful. Maize is characterized by a fast growth and high production of the biomass of shoots (25000 kg/ha×crop) (Huang and Cunningham, 1996), but it does not belong to species efficiently accumulating heavy metals. However, recent studies have shown that chelates added to the soil, among which EDTA is the most effective, can considerably increase Pb accumulation in maize shoots (Huang and Cunningham, 1996; Huang *et al.,* 1997). A similar effect was obtained in the case of lead (Blaylock *et al.,* 1997; Vassil *et al.,* 1998) and cadmium accumulation (Blaylock *et al.,* 1997) by *Brassica juncea*. It was found that the Pb-EDTA complex is taken up more easily by plants and transported through the xylem to shoots.

Higher metal accumulation in shoots after adding chelates to the growth medium can result from increase in their availability for plant roots or from increased/facilitated metal transport to shoots. Metal transport can be easily demonstrated in hydroponic cultures in which almost the whole metal pool is available for plants. The results of such studies can be valuable because it was shown that differences in Cd accumulation in shoots of 19 maize inbred lines did not depend on the kind of cultivation (hydroponic culture or soil), but they were rather of genetic origin (Florijn and Van Beusichem, 1993). Hydroponic cultures are used more and more frequently for fast classification of species able to tolerate and accumulate metals (Cd, Cu, Pb, Zn) in plant shoots (Huang and Cunningham, 1996; Ebbs and Kochian, 1998).

The aim of these studies was to investigate whether EDTA increases Cd accumulation in hydroponically cultivated maize seedlings, and in what way such a modification of the medium affects plant growth.

Material and Methods

Five-day old maize seedlings (*Zea mays* L., Hidosil variety) were transferred into the medium of Hoagland and Arnon (1950) which was continuously aerated during the experiments. Iron was added as Fe-citrate. The plants were grown in a vegetation room at 23/17 °C (d/n) with 16/8 (d/n) illumination at photosynthetic photon flux density of 150 nmol m^{-2} s^{-1}. During 2 days they received Hoagland solution. Then the growth conditions were modified.

Experiments were carried out in the following combinations:

Control plants:	**K**	– Hoagland solution
	K_1	– Hoagland solution + 200 μM EDTA
	K_2	– Hoagland solution + 400 μM EDTA
Cd-treated plants:	**Cd**	– K + 50 μM Cd
	Cd_1	– K_1 + 50 μM Cd
	Cd_2	– K_2 + 50 μM Cd

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Cadmium was added as $Cd(NO_3)_2 \times 4H_2O$, EDTA as $Na_2EDTA \times 2H_2O$. The roots and shoots were analysed 7 and 14 days after application of the metal. The medium was maintained at a constant volume by adding distilled water. Cadmium content was determined by atomic absorption spectrophotometry (Perkin Elmer, model 3300) after wet-ashing of the dry matter (dried at 105 °C) in a $HNO_3/HClO_4$ mixture (4:1, v/v). The leaf area was measured using a GeniScan GS-4500 scanner (Genius, Taiwan), and the area was calculated by computer programme "WITRA" (Warsaw, Poland).

The data for the growth parameters were analysed from 7 replications using 10 seedlings in each. Statistical analyses were calculated using INSTAT 2.0 programme. The differences observed are statistically significant.

Results

Trace amounts of cadmium (up to 0.009 mg/g d.w.) (data not shown) were found in roots and shoots of maize seedlings cultivated in control conditions. In Cd-treated seedlings metal was mostly accumulated in roots (79–94%), and transported in much smaller amounts (6–21%) to shoots (Fig. 1). 200 μM of EDTA added to the medium increased by over two times Cd accumulation in roots after 7-day growth of seedlings in its presence. However, addition of 400 μM EDTA distinctly decreased Cd content in roots and shoots of seedlings treated with 50 μM Cd over 7 days. After 14 days of metal action, both EDTA doses decreased the uptake and accumulation of the metal (Fig. 1). Particularly 400 μM EDTA decreased the Cd level by 65% in roots and by 82% in shoots as compared to seedlings growing without the chelating agent.

Cadmium had toxic effects on seedling growth. The greatest changes induced by the presence of Cd were observed in root growth. After 7 days of the metal treatment their elongation was inhibited by 40%, and after 14 days by 50% in comparison with the control (Fig. 2). A somewhat smaller toxic effect of Cd was observed on the fresh weight of roots (Fig. 3), shoots (Fig. 4) and leaf area (Fig. 5). All these changes were more distinct after 14 days of Cd action. Also EDTA inhibited plant growth, since it reduced most of the growth parameters measured (Figs. 3–5), except root elongation (Fig. 2). The higher was the EDTA dose and the longer the time of its action, the greater was the toxicity of the chelating agent. The highest effect was observed in the leaf area after 14 days of 400 μM EDTA action. At the same time a small chlorosis of leaves was also observed. The determined parameter was by 50% smaller in comparison with the control (Fig. 5). However, it is interesting that EDTA added to the growth medium containing Cd decreased the toxicity of the metal on plant growth, particularly after 14 days (Figs.

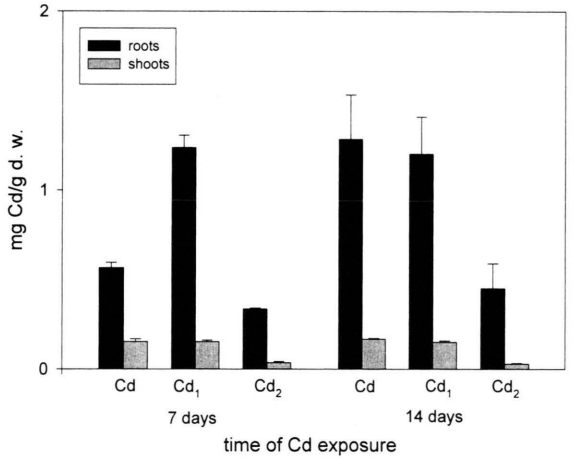

Fig. 1. Cadmium content in roots and shoots of maize seedlings after 7 and 14 days of Cd-treatment. **K** - Hoagland solution, $\mathbf{K}_1$ – Hoagland solution + 200 μM EDTA, $\mathbf{K}_2$ - Hoagland solution + 400 μM EDTA, **Cd** – K + 50 μM Cd, $\mathbf{Cd}_1$ – K_1 + 50 μM Cd, $\mathbf{Cd}_2$ – K_2 + 50 μM Cd. Bars represent mean with standard error.

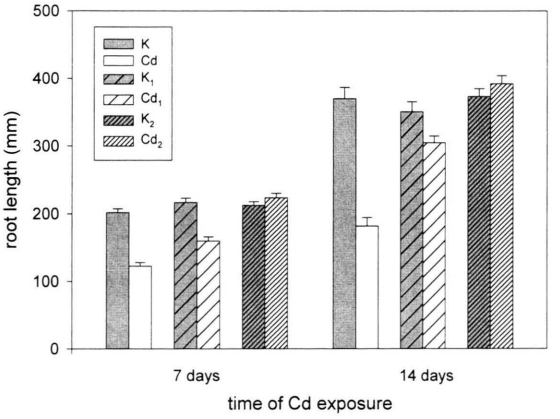

Fig. 2. Effect of Cd and EDTA on root growth of maize seedlings. Explanations as in Fig. 1.

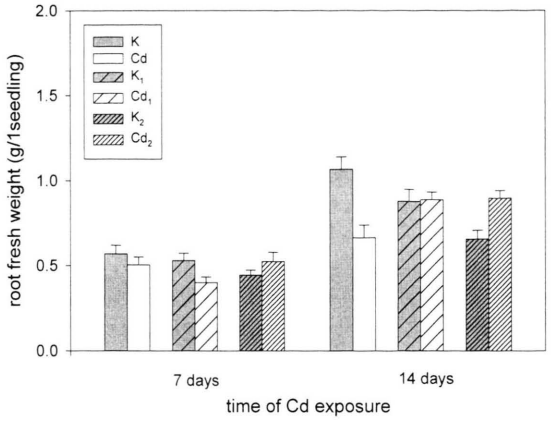

Fig. 3. Effect of Cd and EDTA on fresh weight of roots of maize seedlings. Explanations as in Fig. 1.

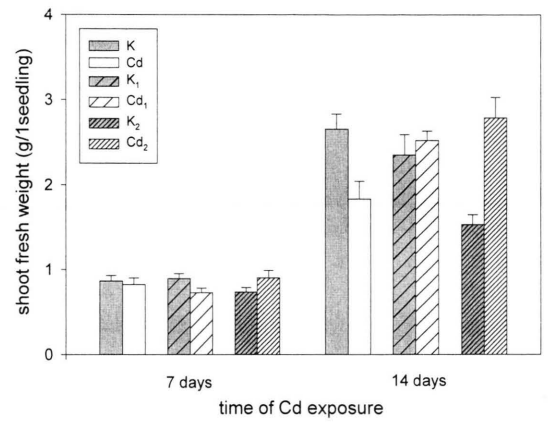

Fig. 4. Effect of Cd and EDTA on fresh weight of shoots of maize seedlings. Explanations as in Fig. 1.

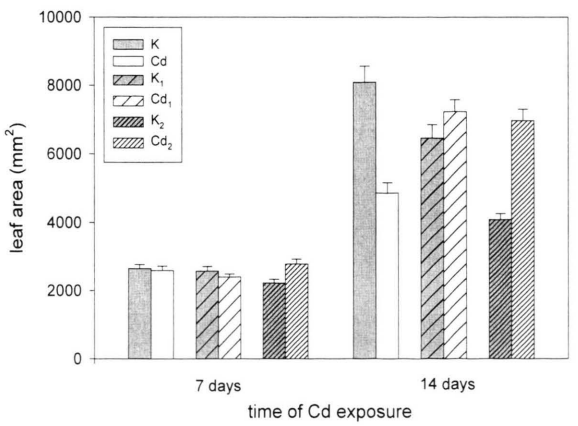

Fig. 5. Effect of Cd and EDTA on leaf area of maize seedlings. Explanations as in Fig. 1.

2–5). This effect was most distinct in the case of root elongation, which in the presence of Cd and 400 μM EDTA was even higher than that of control (K – without EDTA) plants (Fig. 2). Changes of dry weight showed a similar tendency as those of root and shoot fresh weight (data not shown).

Discussion

In our earlier studies, the Cd toxic effect on growth parameters of maize plants as well as a possible Cd detoxication by phytochelatins were tested. Cd concentrations below 50 μM were found to have no influence on the parameters studied, or it was only slight after 7 days of Cd action. However, 50 μM Cd greatly induced the accumulation of phytochelatins, particularly in roots.

Retention of Cd in roots is considered as a mechanism of metal tolerance and it is a common phenomenon in many plant species (Jarvis *et al.*, 1976; Jastrow and Koeppe, 1980; Hardiman and Jacoby, 1984; Greger and Lindberg, 1986) including maize (Florijn and Van Beusichem, 1993; Lozano-Rodriguez *et al.*, 1997). In our studies roots accumulated 79–94% of the metal taken up (Fig. 1). Similar accumulation of Cd (80%) in roots was also observed by Lozano-Rodriguez *et al.* (1997). The cause of high accumulation in roots can be binding of the metal in cell walls (Khan *et al.*, 1984), chelating by organic acids and phytochelatins in the cytoplasm and vacuoles of root cells (Punz and Sieghardt, 1993), or limited xylem transport to the shoots (Hart *et al.*, 1998).

In the case of Pb it was shown that both the uptake and transport of the metal to shoots can be increased by using synthetic chelates, *e.g.* EDTA. Pb-EDTA chelates penetrate cell membranes more readily and in such form are transported by xylem to shoots (Blaylock *et al.*, 1997; Huang *et al.*, 1997; Vassil *et al.*, 1998). This was also confirmed by Huang *et al.* (1997), who showed that under the influence of EDTA Pb accumulation in maize shoots increased 120 times in 24 h as compared with control. These observations are inconsistent with the reports that absorption and transport of synthetic chelates or their complexes with divalent metals are obstructed (DeKock and Mitchell, 1950).

Unfortunately, maize is not so effective in Cd accumulation. Addition of EDTA to the medium,

particularly 400 μM, induced a considerable decrease of the metal uptake as well as of its accumulation in roots and shoots (Fig. 1). The ratio of the metal accumulation in roots to that in shoots was hardly affected; this indicates that EDTA is not a factor facilitating translocation of Cd in maize plants. The effect of EDTA depressing Cd uptake from aqueous solutions was also observed in the case of bush beans (Hardiman and Jacoby, 1984), sugar beets (Greger and Lindberg, 1986) and duckweed (Nasu and Kugimoto, 1983), though in the latter species chelates facilitated in some cases the metal uptake (Polar and Küçükcezzar, 1986). It is probably the effect of chelating Cd^{2+} by EDTA in the solution and of obstructed uptake of such complexes by plants, similarly as complexes of other divalent metals (DeKock and Mitchell, 1950).

Reduction of various plant growth parameters under the influence of Cd or EDTA was described by several authors. In our studies Cd decreased all the growth parameters investigated; its effect depends on the time of metal action. The greatest and earliest changes caused by Cd toxicity were observed in root elongation. This parameter is most frequently used for determination of Cd toxicity (Prasad, 1995). A 30–37% reduction of the fresh weight of maize roots and shoots observed in our studies after 14-day Cd treatment is comparable with the results of Lozano-Rodriguez *et al.* (1997). The opinion of most of the authors is that frequently growth reduction is the consequence of disturbed cell division and elongation, mineral nutrition, water relations, photosynthesis, respiration and protein metabolism caused by cadmium (for references see Punz and Sieghardt, 1993; Prasad, 1995).

In our experiments, EDTA added to the growth medium of maize seedlings did not inhibit elongation of roots (Fig. 2), but they were thinner and had a lower number of laterals which was the reason for fresh weight decrease (Fig. 3). Greger and Lindberg (1986, 1987) observed that EDTA considerably reduced the root length of sugar beet seedlings, but more laterals developed and the root mass increased in relation to that of the whole plant. At the same time they described a decrease of the fresh weight of shoots and leaf area under the influence of EDTA, which is in agreement with our results (Fig. 4 and 5). Vassil *et al.* (1998) estimated EDTA phytotoxicity by changes of water content in shoots of Indian mustard seedlings, which in severe cases resulted in visible drying of the tissue and formation of necrotic lesions. EDTA toxicity is considered to be caused probably by interference with plant mineral nutrition. Uncoordinated EDTA would bind various essential divalent cations (Fe^{2+}, Zn^{2+}, Cu^{2+}) disturbing important cellular metabolic processes (Greger and Lindberg, 1987; Polar and Küçükcezzar, 1986; Vassil *et al.*, 1998).

Chelates, however, may protect plants from damages caused by heavy metals. Decrease of cadmium toxicity by EDTA was described by Polar and Küçükcezzar (1986) for relative growth rates of duckweed and also by Greger and Lindberg (1986 and 1987) in the case of sugar content and growth of sugar beet. In the latter case toxicity symptoms depended on Cd and EDTA concentration in the nutrient medium. It was also shown in our studies that the toxic Cd effect was supressed by EDTA on every growth parameter examined (Figs. 2–5). This seems to be due to limited metal uptake by plants (as it appears from the data presented in Fig. 1). Polar and Küçükcezzar (1986) however concluded from their observations that the detoxificating effect of EDTA cannot be always interpreted in this way.

Surprising is the fact, why the growth of maize seedlings was so good when 50 μM Cd and 400 μM EDTA combination in the medium was used. Although the whole Cd pool present in the medium is bound by EDTA (Cd : EDTA = 1 : 1) and the uptake of such complex by plants is obstructed, toxicity symptoms due to EDTA excess should be observed in them. These symptoms should be more distinct than in the presence of 200 μM EDTA (K_1).

Cd concentration available for plants in solution of soils from areas contaminated with this metal ranges from 300–400 μg/L, i.e. about 3.5 μM (Kabata-Pendias and Pendias, 1993). Therefore, the results of our laboratory studies on Cd toxicity and tolerance in maize seedlings cannot be related to natural conditions. EDTA application in situ should be reasonable because of possible multiple and toxic action of this chelate in the soil. It can not only increase the availability of toxic metals for plants but also change the balance between other essential ions

in the medium. Moreover, if EDTA is applied as a salt the introduction into the medium of large amounts of other ions (e.g. K, Na, Ca, Mg), which could cause a salinity stress, should be taken into account.

The EDTA doses we used were very high and appeared very toxic for plants. Following these preliminary studies, our intention is to find the lowest EDTA dose effective for Cd detoxication in maize plants.

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