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BRIEF COMMUNICATION

Lead uptake, toxicity and accumulation in *Phaseolus vulgaris* plants

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Abstract

The effects of lead were investigated in bean plants (*Phaseolus vulgaris* L. cv. Zlota Saxa) grown hydroponically in nutrient solution and exposed to $Pb(NO_3)_2$ (0.1, 0.5, 1 mM) with or without equimolar concentrations of chelator ethylenediaminetetraacetic acid (EDTA). The roots treated only with $Pb(NO_3)_2$ accumulated up to 25 g(Pb) kg^{-1}(d.m.), during 4-d exposure. However, in bean plants exposed to 0.5 mM Pb + 0.5 mM EDTA or 1 mM Pb + 1 mM EDTA 2.5 times less Pb was determined. In bean plants treated only with Pb, less than 6 % of total lead accumulated was transported to the aboveground parts, while in the case of plants grown with Pb + EDTA, around 50 % of total Pb was transported to the shoots.

Additional key words: bean, EDTA, hydroponics, lead translocation.

The present research focuses on such plants as Indian mustard, maize, pea, oats or barley, which exhibit tolerance to environment contaminated with heavy metals and, simultaneously, show high biomass (Huang *et al*. 1998, Ebbs and Kochian 1998, Wu *et al*. 1999). In order to raise the relatively low level of metal uptake by these plants, some authors suggest the use of synthetic chelators. Chelates have been shown to desorb heavy metals from the soil matrix into soil solution, facilitate metal transport into the xylem, and increase metal translocation from roots to shoots of fast-growing plants (Begonia *et al*. 2005). It was shown that among the studied compounds, EDTA (ethylenediaminetetraacetic acid), DTPA (diethylenetriaminepentaacetic acid), CDTA (cyclohexanediaminetetraacetic acid) and EGTA (ethyleneglycolbis-(beta-amino-ethylether)-N,N'-tetraacetic acid), the first one is the most effective Pb ion chelator (Huang *et al*. 1997). Due to its application, 150-fold increase in Pb accumulation was observed in the aboveground parts of Indian mustard growing on soil containing 600 mg(Pb) kg^{-1}(d.m.) (Blaylock *et al*. 1997), whereas 120-fold for maize on soil containing 2.5 g(Pb) kg^{-1}(d.m.) (Huang *et al*. 1997). It was determined that EDTA not only increases the amount of Pb taken up by plants from the soil, but it also increases the volume of metal transport through the xylem and Pb translocation from roots to shoots and leaves (Huang *et al.* 1997, Epstain *et al*. 1999). However, the application of EDTA may increase the potential off-site migration of metals, disturbed nutrient balance, moreover EDTA and EDTA-HM complexes can be toxic to plants and microorganisms.

From our earlier investigations (Piechalak *et al*. 2002) we found that among three examined legume plants, *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris*, roots of bean plants accumulated the highest amount of lead. Moreover, most of metal were adsorbed on the surface of the bean roots. The aim of the experiments presented in this paper is to consider the possibility of growing *Phaseolus vulgaris* cultivars with enhanced ability to accumulate Pb in the aboveground parts of plants as a result of adding synthetic chelator (EDTA) and possibility to use these plants for phytoremediation.

Phaseolus vulgaris L. (cv. Zlota Saxa) seedlings were cultivated as described earlier (Tomaszewska *et al* 1996, Piechalak *et al*. 2002, 2003). After 3-d, a 100 × diluted Hoagland solution replaced the medium and the following were added: *1*) 0.1 mM $Pb(NO_3)_2$, *2*) 0.1 mM $Pb(NO_3)_2$ + 0.1 mM EDTA, *3*) 0.5 mM $Pb(NO_3)_2$, *4*) 0.5 mM $Pb(NO_3)_2$ + 0.5 mM EDTA, *5*) 1 mM $Pb(NO_3)_2$, *6*) 1 mM $Pb(NO_3)_2$ + 1 mM EDTA, *7*) 0.5 mM

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Abbreviations: EDTA - ethylenediaminetetraacetic acid, HM - heavy metal.
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EDTA, and control was without any addition. The plant material was collected, washed in 10 mM $CaCl_2$, then in bidistilled water. The samples were frozen in liquid nitrogen and stored at -80 °C or directly used.

The index of tolerance (IT) was determined according to the Wilkins (1957). The uptake of lead ions from Hoagland nutrient medium and content of Pb in leaf tissue was measured with the use of the atomic absorption spectrometry as described earlier (Piechalak *et al*. 2003). The accuracy of the used method was checked using a certified reference material (CL-1CRM, cabbage leaves) and the aqueous standards (Baralkiewicz *et al.* 2004). The statistical analysis of the results was done with the use of the statistic software *Statistica v.6*, counting arithmetic means and mean errors. The results of growth parameters are mean values of 21 - 25 measurements from 3 - 4 independent repetitions. The measurement of Pb contents in different plant organs and Pb uptake from the nutrient solution were done 3 times.

The presence of Pb in medium caused visible changes in the development of *Phaseolus vulgaris* plants. After 4 d the plants showed an inhibition of root elongation, a decrease in the number of hair roots, decrease in the production of fresh mass and partial inhibition of growth of the aboveground parts. These changes were especially visible at the highest 1 mM concentration of $Pb(NO_3)_2$. The application of Pb with EDTA led to inhibition of the Pb phytotoxicity effect on the examined plants. EDTA supplementation caused a decrease in the inhibition of root elongation growth, increase in the number of hair roots, diminished browning and sliming of roots. These differences resulted in the higher fresh mass (71 % of the control plants at Pb + EDTA while 61 % at only 1 mM Pb). The value of IT for plants exposed to 1 mM $Pb(NO_3)_2$ and to 1 mM $Pb(NO_3)_2$ + EDTA was 22 and 65 %, respectively. Moreover, differences of IT values and fresh mass between plants grown with lower concentration of Pb (0.5, 0.1 mM) and Pb + EDTA (Table 1) were found. The influence of EDTA alone was also checked. After 4-d exposure to 0.5 mM EDTA, the IT was 89 %, and the fresh mass was 93 % of control, which allowed us to draw a conclusion that supplementation of the chelator only slightly affects the development of *P. vulgaris* plants.

An inhibition of root elongation growth of bean confirms the results obtained earlier by many other researchers. The negative effect of 1 mM Pb on bean, lupin, pea and broad bean were described in our earlier reports (Tomaszewska *et al*. 1996, Piechalak *et al*. 2002, 2003). Geebelen *et al*. (2002) also showed a visible reduction of bean root length already at 0.08 mM $Pb(NO_3)_2$, whereas the application of 0.4 mM $Pb(NO_3)_2$ decreased fresh mass of bean roots by 90 %. The mechanisms leading to a decrease in root elongation growth are not well known. This must be a complex process, which involves participation of disturbed water balance of plants (Wierzbicka *et al*. 1994), inhibition of cytokinesis, disturbances of c-mitoses, or a decrease in the number of mitotic divisions in the meristematic zone of roots (Przymusinski *et al*. 1991). It was shown that lead as other metals (Cd, Cu, Zn) also caused inhibition of morphogenesis (Agrawal and Sharma 2006). Un unfavourable effect of EDTA and EDTA-HM complexes on the development of treated plants and the amount of their dry mass was observed by Jiang *et al*. (2003), Cui *et al*. (2004), and Chen *et al*. (2004), probably due to disorders in the balance of minerals, such as Zn, Cu, Fe or Ca, which further disturbs cell metabolism and destabilize biological membranes.

It was determined that the bean plants grown with Pb + EDTA, accumulated much smaller amounts of Pb than plants exposed only to Pb. The amounts of Pb accumulated was 3.5, 4 and 80 times less in roots of plants grown with 1, 0.5 and 0.1 mM Pb + EDTA, respectively than in roots of plants at Pb only (Table 1).

During 4-d exposure to 0.1 and 0.5 mM $Pb(NO_3)_2$ and to 0.1 or 0.5 mM $Pb(NO_3)_2$ + EDTA the amount of Pb in the nutrient solution decreased by 78 and 82 %, and by 30.5 and 57.8 %, respectively. When the highest 1 mM concentration of lead nitrate was used, the Pb content during 24 h decreased by 72 % and for 1 mM Pb+ 1 mM EDTA by only 26 %.

Like most plants, bean accumulates Pb mainly in roots and only small part of the metal taken up by the plant is transported to its aboveground parts. Participation of aboveground parts in the total amount of metal

Table 1. The index of tolerance (IT), fresh mass and Pb content in the *Phaseolus vulgaris* plants grown in the presence of Pb or Pb + EDTA for 4 d. Means ± SE, $n = 4$.

Treatment	IT [%]	Fresh mass [g plant^{-1}]	Pb content [g kg^{-1}(d.m.)] roots	stems	leaves
Control	100 ± 10.0	1.59 ± 0.25	0.07 ± 0.005	0.007 ± 0.002	0.002 ± 0.0005
0.1 mM Pb	87 ± 6.0	1.50 ± 0.21	13.66 ± 2.300	0.327 ± 0.056	0.042 ± 0.002
0.1 mM Pb + 0.1 mM EDTA	96 ± 9.3	1.47 ± 0.19	0.20 ± 0.056	0.169 ± 0.021	0.148 ± 0.013
0.5 mM Pb	58 ± 5.9	1.12 ± 0.20	18.05 ± 3.560	1.003 ± 0.240	0.053 ± 0.006
0.5 mM Pb + 0.5 mM EDTA	73 ± 8.5	1.30 ± 0.15	5.12 ± 1.250	2.038 ± 0.650	0.976 ± 0.056
1.0 mM Pb	25 ± 2.5	0.97 ± 0.11	25.74 ± 2.300	1.508 ± 0.950	0.070 ± 0.006
1.0 mM Pb + 1.0 mM EDTA	65 ± 7.3	1.14 ± 0.13	7.21 ± 1.890	5.670 ± 1.020	3.510 ± 0.680

accumulated by the whole bean plant was 2.7 to 5.8 %, out of which only less than 0.5 % of the total amount of Pb taken up was accumulated in the leaves (Table 1).

The addition of the chelator significantly affected the participation of particular organs in Pb accumulation. After 4-d exposure to 0.5 mM Pb + EDTA and 1 mM Pb + EDTA, over 2 times more Pb was shown in stems of the studied plants than in plants grown only with Pb, which constituted, respectively 27 % and 35 % of the total Pb accumulated by whole plant. The addition of EDTA also increased metal transport to the leaves. It was shown that 20 and 30 times more Pb was accumulated in the leaves of plants grown with 0.5 mM Pb + EDTA and 1 mM Pb + EDTA, which constitutes 14.7 and 21.4 %, respectively, of the total Pb accumulated by the plants (Table 1).

In plants exposed to Pb + EDTA the participation of aboveground parts in the accumulation of metal significantly increased, after 4-d treatment with 0.1 mM Pb + EDTA, 0.5 mM Pb + EDTA and 1 mM Pb + EDTA the contents of Pb in the shoots were 0.317, 3.144, and 9.176 g(Pb) kg^{-1}(d.m.), respectively, which constitutes 66, 42, and 56 % of the total amount of lead accumulated by bean plants.

It is known that metal ions penetrate plants mainly through roots. It was found that metal uptake is at first stopped on the root surface, then, a large portion of metal penetrates roots and is bound in the cell wall forming insoluble deposits there, while some part of it is stored in intercellular spaces (Antosiewicz 2004, Lou *et al*. 2004, Liu *et al*. 2007, Malecka *et al*. 2008). It was also determined that celluloses and lignin bound substantial amount of lead (Marmiroli *et al*. 2005). Our earlier research showed that, irrespective of the applied concentration of lead nitrate, over 90 % of the total metal uptake was accumulated in roots (Piechalak *et al*. 2002). In present experiments with *P. vulgaris*, we confirmed that roots are the main site of Pb^{2+} accumulation (Table 1).

Only few authors observed that effect of chelator application. Jiang *et al*. (2003) evidenced a similar effect on Cd accumulation in Indian mustard. In the roots of plants exposed to Cd + EDTA, the metal accumulation declined when compared to the roots of plants grown only in the presence of cadmium nitrate. Similarly, the rape plants grown with 1 and 3 mM $Pb(NO_3)_2$ + EDTA for 14 d showed a decrease in Pb accumulation by the whole plants (Wasinkiewicz 2007).

In the most papers it has been reported that Pb accumulation was greater after EDTA application for example in Indian mustard (Vassil *et al*. 1998, Epstein *et. al*. 1999), maize (Huang *et al*. 1998, Wu *et al*. 1999), and bean (Sarret *et al*. 2001). Krishnamurti *et al.* (1997) and Wu *et al.* (2003) proved that EDTA application resulted in larger amounts of these metals in soil solution: 300 times for Cd, 600 times for Pb, 100 times for Cd and 30 times for Zn. These authors claimed that Pb was taken up in the form of complexes with EDTA and in this form it penetrated plants more easily (Vassil *et al*. 1998, Wu *et al*. 1999).

However, the application of EDTA caused significant increase in the degree of Pb translocation to the aboveground parts of bean plants. In agreement with our results, Huang *et al*. (1997) obtained over 100-fold increase of the amount of Pb in the aboveground tissues of pea, but amount of Pb in the stems and leaves of pea was over 3 times higher than in maize. Similarly, Chen *et al*. (2004) showed for 10 plants species (6 dicotyledonous and 4 monocotyledonous ones) that EDTA boosted the level of Pb transport after 2 d of treatment even 100 times. The effect of EDTA treatment on Pb uptake, accumulation and translocation to the aboveground parts of plants depends on plant species, age, state of development and also on metal bioavailability.

In recent years numerous reports have been published on the application of synthetic chelators to enhance uptake and transport of heavy metals by plants for phytoremediation purposes. The observed disadvantageous effect of EDTA on the total Pb accumulation by the whole plants during a short treatment should be further investigated in order to determine the mechanism responsible for the decrease of total Pb accumulation in bean plants. However, increased Pb content in aboveground parts, is a crucial factor for phytoremediation.

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