Original Research

Accumulation of Pb and Cd and its Effect on Ca Distribution in Maize Seedlings (*Zea Mays* L.)

E. Małkowski[1]*, R. Kurtyka[1], A. Kita[2], W. Karcz[1]

[1]Department of Plant Physiology, Faculty of Biology and Environmental Protection, University of Silesia, 28 Jagiellońska St, 40-032 Katowice, Poland
[2]Department of Analytical Chemistry, University of Silesia, 9 Szkolna St, 40-006 Katowice, Poland

Received: 29 June, 2004
Accepted: 10 August, 2004

Abstract

In the present study the accumulation of Pb and Cd in maize seedlings (*Zea mays* L., cv. K33 x F2) and its effect on the content of Ca in their organs were investigated. The influence of both metals on dry weight yield was also studied. Concentrations of Pb, Cd and Ca were measured in 15 mm-long apical root segments as well as in whole mesocotyls and coleoptiles after 24 h of incubation of seedlings in hydroponic solutions. It was observed that in Pb- or Cd-treated maize seedlings the dry weight of root apical segments was markedly increased, whereas in the shoots it was less affected. It was also found that the content of both metals in maize seedling organs rose with increasing concentrations of Pb or Cd in the hydroponic solutions. The highest concentrations of both metals were observed in roots, in which the accumulation of Pb was 10-fold higher than that of Cd. By contrast, higher accumulation of Cd than Pb was found in mesocotyl and coleoptile. These data suggest that the mechanisms of Pb and Cd translocation from roots to shoots are different and that the accumulation of Ca in roots and its translocation to shoots was less affected by Pb than Cd.

Keywords: Pb and Cd, Ca accumulation, roots, mesocotyls, coleoptiles, *Zea mays* L.

Introduction

Heavy metals are present in soils as natural components or as a result of human activities. Metal-rich mine tailings, metal smelting, electroplating, fossil fuel combustion, intensive agriculture, and waste disposal on land are the most important human activities that contaminate soils with large quantities of the toxic metals [1]. Among toxic metals, Pb and Cd appear to be the most dangerous to the environment.

These non-essential elements are taken up through the roots of many plant species and accumulated in all plant organs. Taken up in excess, Pb and Cd can cause deficiencies or adverse ion distribution within the plant [2, 3, 4, 5, 6], disturb metabolism of nucleus [7], photosynthesis [8] or reduce rate of growth [9, 10, 11].

*Corresponding author; e-mail: malkowsk@us.edu.pl

Many researchers have investigated the uptake and accumulation of Pb and Cd in different plant species [2, 12, 13, 14, 15, 16]. However, the mechanism of accumulation of heavy metals is still not completely understood.

Among crop plant species, maize is one of the most important and is grown throughout the world. It also can be used successfully in phytoextraction, which is a new technology for removing heavy metals from contaminated soils [17]. The uptake of Pb and/or Cd [13, 18, 19, 20] and effects of both metals on mineral nutrition [21, 22] in maize have been studied previously. These experiments were conducted with maize plants older than 10 days, whereas information concerning the uptake of Pb and Cd and their interaction with other ions at the early stages of maize seedling development is poorly documented. For example, Wierzbicka and Obidzińska [23] conducted experiments on the effect of Pb on imbibition and germination of maize seeds, but they did not measure uptake of Pb or Ca.

It was reported that Pb and Cd uptake affected accumulation of Ca in organs of various plant species [3, 16, 24, 25, 26, 27]. Based on these observations, it was postulated that the heavy metals could compete with Ca for entry into plant cells and affect accumulation and distribution of Ca in plants [16, 24, 26, 28]. Recently, it has been documented that uptake of Pb and Cd by plant cells is mediated by Ca^{2+} channels and/or carriers and disturbs accumulation of Ca [17, 29].

In the present work the accumulation of Pb and Cd and its effect on Ca distribution in roots, mesocotyls and coleoptiles of maize seedlings at their early stages of development were investigated. The influence of both metals on dry weight yield was also studied.

Material and Methods

The experiments were carried out with etiolated 3-day-old maize seedlings (*Zea mays* L., cv. K33 x F2) that were transferred to hydroponic containers filled with control solution of the following composition: KCl (1 mmol dm^{-3}), NaCl (0.1 mmol dm^{-3}) and $CaCl_2$ (0.1 mmol dm^{-3}) or to the solution with the same salt composition, containing additionally $PbCl_2$ or $CdCl_2$ at a final concentration of 0.1 mmol dm^{-3} or 1.0 mmol dm^{-3}. The control solution with the salt composition mentioned above is used routinely in our laboratory [30, 31]. The initial pH of the solutions was adjusted to 5.8-6.0 with 0.1 N NaOH or 0.1 N HCl. The plants were grown in the solution in the dark at 27ºC for 24 h. After this time roots were rinsed 2-fold with distilled water and blotted dry with tissue paper. Then 15 mm apical segments of seminal roots, whole mesocotyls and coleoptiles (with the first leaf removed) were sampled and the plant tissue was dried at 80°C for 3 days to determine its dry weight. In the current study the apical root segments, instead of whole roots, were used to asses the effect of Pb and Cd on Ca accumulation and distribution, because the uptake of Ca and its translocation to shoots is much higher in apical than in basal root zones [32].

For metal analyses, dry plant tissue was digested with ultra-pure concentrated nitric acid (Merck). The concentrations of Pb, Cd and Ca were measured by emission spectrometry with excitation by an argon inductively-coupled plasma technique (ICP-AES). Standard solutions of metals (Merck) were used as reference. All chemicals were of analytical grade. All experiments were replicated at least four times, with 100 seedlings in each replicate.

Data were analyzed using the computer software Statistica for Windows, version 5.1 ´97, StatSoft Inc., Tulsa, USA (Licence – SP711660411NET5). Differences between individual treatments and control were analyzed using one-way ANOVA and LSD test. Statistical significance was defined at $p < 0.05$.

Results

As can be seen in Fig. 1 the dry weight of the 15 mm long apical root segments was almost twofold greater, as compared to the control (100 %), when maize seedlings were exposured to both 0.1 mmol dm^{-3} and 1.0 mmol dm^{-3} Pb. However, mesocotyl and coleoptile dry weight did not significantly differ, as compared to the control solution, in plants treated with 0.1 mmol dm^{-3} or 1.0 mmol dm^{-3} Pb (Fig. 1). In turn, when maize seedlings were treated with 1.0 mmol dm^{-3} Cd an increase of dry weight of both root segments and mesocotyls was observed (Fig. 2). By contrast to Pb, Cd at 1.0 mmol dm^{-3} decreased dry weight of coleoptiles. However, in plants treated with Cd large variations in dry weight yields were observed and differences among means of dry weight of control and Cd-treated plants were not statistically significant (Fig. 2).

The data in Table 1 indicate that accumulation of Pb and Cd in maize seedling organs increased with increasing concentrations of both metals in the hydroponic solutions. The content of Pb in root segments, at both concentrations of heavy metals used, was approximately tenfold higher as compared to Cd. The data in Table 1 also suggest that transport of Cd from roots to shoots was less restricted than that of Pb and its level in mesocotyl and coleoptile at 0.1 mmol dm^{-3} was markedly higher than that of Pb. Plants from 1.0 mmol dm^{-3} treatments had significantly higher concentration of Cd than Pb in coleoptile but there was no statistically significant difference in concentration of both metals in mesocotyls.

Figure 3 shows a comparison of the effects of both metals and the control treatment on accumulation of Ca in seedling organs. The data indicate that following exposure of seedlings to Pb at both concentrations (0.1 mmol dm^{-3} and 1.0 mmol dm^{-3}), the accumulation of Ca in root segments was enhanced 2- to 2.7-fold as compared to

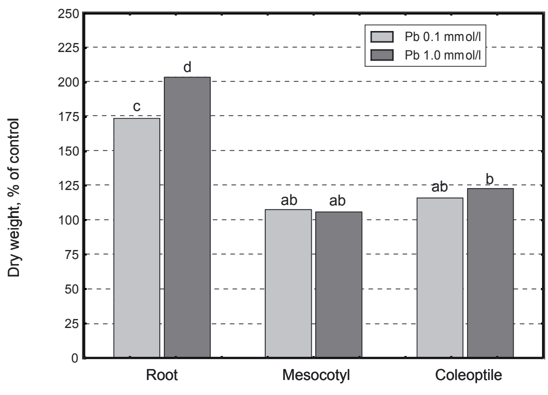

Fig. 1. Effects of Pb on dry weight (mg) of maize seedling organs (*Zea mays* L.), shown as percent of control. Values are means (n = 4). Columns marked with the letter "a" are not significantly different from the control using LSD test ($p < 0.05$). Columns marked with the same letter are not significantly different from each other using LSD test ($p < 0.05$).

Table 1. Effects of different concentrations (0.1 and 1.0 mmol/l) of Pb or Cd in the hydroponic medium on concentrations (mg/kg d.w.) of both metals in the organs (root, mesocotyl, coleoptile) of maize seedlings. Values are means ± SE (n=4).

Concentration of metals in the hydroponic medium	Plant organ		
	Root	Mesocotyl	Coleoptile
Cd 0.0 mmol/l (control)	1.8 ± 0.32 a	0.5 ± 0.08 a	1.2 ± 0.37 a
Cd 0.1 mmol/l	4621.3 ± 630 b	91.5 ± 11.6 b	25.5 ± 5.7 b
Cd 1.0 mmol/l	10,010.5 ± 650 c	140.0 ± 33.0 b	70.0 ± 6.0 c
Pb 0.0 mmol/l (control)	5.2 ± 0.20 d	0.3 ± 0.03 a	1.0 ± 0.13 a
Pb 0.1 mmol/l	40,293.3 ± 1261 e	52.0 ± 9.2 c	11.0 ± 4.5 d
Pb 1.0 mmol/l	113,404.7 ± 9532 f	113.3 ± 7.0 b	39.0 ± 7.5 b

Means followed by the same letter in a column are not significantly different from each other using the LSD test ($P < 0.05$).

control solution. In plants treated with 0.1 mmol dm^{-3} Cd, the concentration of Ca was 3-fold greater than in the control. Contrary to Pb, Cd at higher concentration (1.0 mmol dm^{-3}) decreased content of Ca by 30%, as compared to the control, but the difference was not statistically significant (Fig.3A).

In mesocotyls the effect of Pb on Ca accumulation showed no definite tendency, whereas Cd decreased the Ca content in these organs (Fig. 3B). In coleoptiles Pb and Cd had no statistically significant effect on Ca concentration (Fig. 3C).

Discussion

The data presented in this paper show that dry weight of the 15 mm long apical root segments was enhanced, as compared to the control, by exposure of maize seedlings to both Pb concentrations, whereas dry weight of mesocotyls and coleoptiles was not significantly affected

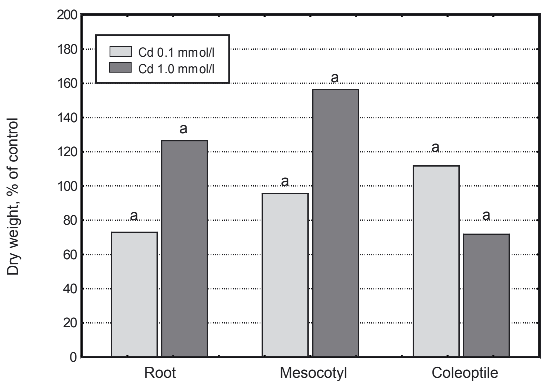

Fig. 2. Effects of Cd on dry weight (mg) of maize seedling organs (*Zea mays* L.), shown as percent of control. Values are means (n = 4). Columns marked with the letter "a" are not significantly different from the control using LSD test ($p < 0.05$). Columns marked with the same letter are not significantly different from each other using LSD test ($p < 0.05$).

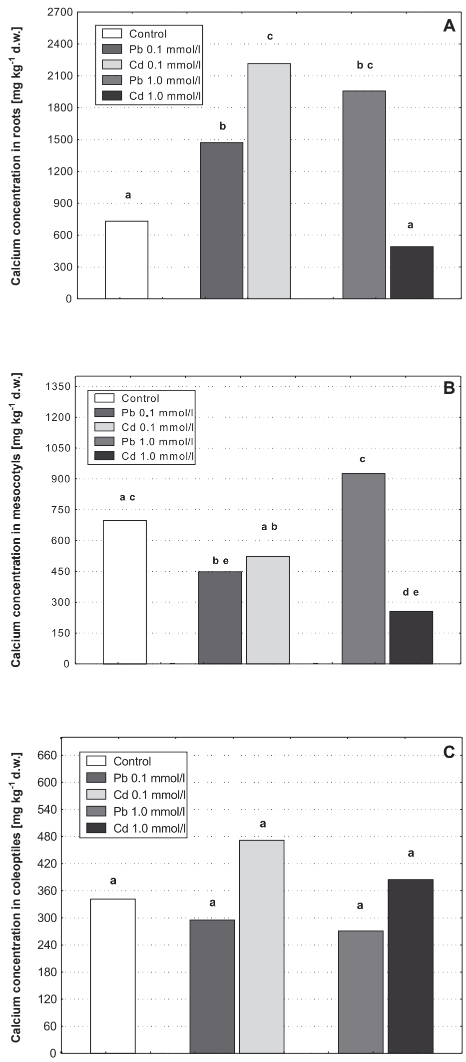

Fig. 3. Effects of Pb and Cd on accumulation of Ca in maize (*Zea mays* L.) seedling organs:
A – concentration of Ca in roots; B − concentration of Ca in mesocotyls; C – concentration of Ca in coleoptiles. Values are means (n = 4). Columns marked with the same letter are not significantly different from each other using LSD test ($p < 0.05$).

(Fig. 1). Similar results have recently been demonstrated by Małkowski et al. [27], who showed that in maize seedlings exposed to Pb (at 0.1 mmol dm^{-3} and 1.0 mmol dm^{-3}) the dry weight of root apical segments (8 mm) was markedly increased, whereas the dry weight of shoots was not significantly affected. In the case of Cd-treated maize seedlings (Fig. 2) the higher dry weight of roots and mesocotyls (statistically insignificant) was found at 1.0 mmol dm^{-3}. By contrast to roots and mesocotyls, dry weight of coleoptiles was not changed by Cd (Fig. 2). However, there is no data concerning the effect of Cd on dry weight of maize seedlings at their early stages of development. Because of different experimental conditions (e.g. concentration of Cd, time of exposure, seedlings growth stages), direct quantitative comparison of our data for Cd with the results presented by other authors [22, 33, 34] is difficult. The higher dry weights of root apical segments, in the presence of Pb or Cd, found in the present study, may have been due to the fact that roots in plants treated with both metals were shorter but thickened (data not shown).

The data on accumulation of Pb and Cd have clearly shown that both metals were strongly accumulated in roots, but substantially lower amounts were observed in mesocotyls and coleoptiles. It was also found here that accumulation of Pb in maize root apical segments was 10-fold higher as compared to Cd (Table 1). These results are in agreement with the data obtained by other authors for different plant species [2, 5, 15, 28]. It was proposed by Brennan and Shelley [35] that very high accummulation of Pb in roots might be connected with prepicitation of the metal at the root surface as amorphous Pb-phosphate. It is noteworthy that transport of Cd to shoots was higher as compared to Pb (Table 1). These data suggest that mechanisms of translocation of Pb and Cd from roots to shoots are different. Similar results for translocation of Pb and Cd were also reported by Burzyński [2] and Kim et al. [28] in experiments with *Cucumis sativus* and *Oryza sativa*, respectively. Since the transport of Ca^{2+} ions by channels or transporters is inhibited by Pb and Cd [17, 29], it would be expected that the accumulation of Ca in the presence of the heavy metals should be reduced. As indicated from results presented here this was not the case, because increased Pb accummulation in root tips (Table 1), was connected with higher uptake of Ca (Fig. 3A).

Wierzbicka [36] has recently reported that Pb stimulated thickening of the cell walls in roots, which was a result of the synthesis of new cell wall polysaccharides. This new cell wall should create new binding sites for divalent cations and enhance their accumulation in roots. Results shown in the present study might suggest that accumulation of Pb and Ca in maize root apical segments might be related to binding of both divalent cations to the newly synthesised cell wall. Accumulation of Pb in plant cell walls was also observed by other authors [36, 37, 38].

Cadmium at higher concentration (1.0 mmol dm^{-3}) decreased the content of Ca in root segments by 30% as compared to the control (not statistically significant) (Fig.3A). These data suggest that Cd, contrary to Pb, should compete with Ca for entry to the root cells.

Results presented in the current study showed that accumulation of Ca in roots and its translocation to shoots were less affected by Pb than Cd. It is proposed that this phenomenon is related to binding of higher amount of Pb, as compared to Cd, in the root apoplast (cell walls). Cadmium, which is not bound in the apoplast could compete with Ca for transport systems. Thus, we conclude that effects of Cd on accumulation and distribution of Ca in maize seedlings, contrary to Pb, might be caused via competition for the transport systems across the plasma membrane but not via competition for the binding sites on the cell wall.

References

1. ROSS S.M. Sources and forms of potentially toxic metals in soil-plant systems. In: Ross SM [ed.], *Toxic metals in soil-plant systems*, pp. 3-25. John Wiley & Sons Ltd, Chichester, West Sussex, England, **1994.**
2. BURZYŃSKI M. The influence of lead and cadmium on the absorption and distribution of potassium, calcium, magnesium and iron in cucumber seedlings. Acta Physiologiae Plantarum **9,** 229, **1987**.
3. ANTOSIEWICZ D.M. Mineral status of dicotyledonous crop plants in relation to their constitutional tolerance to lead. Environmental and Experimental Botany **33,** 575, **1993.**
4. SIEDLECKA A. Some aspects of interactions between heavy metals and plant mineral nutrients. Acta Societatis Botanicorum Poloniae **64**, 265, **1995**.
5. BURZYŃSKI M., BUCZEK J. Uptake and assimilation of ammonium ions by cucumber seedlings from solutions with different pH and addition of heavy metals. Acta Societatis Botanicorum Poloniae **67**, 197, **1998**.
6. PÄIVÖKE A.E.A. Soil lead alters phytase activity and mineral nutrient balance of *Pisum sativum*. Environmental and Experimental Botany **48,** 61, **2002.**
7. GABARA B., KRAJEWSKA M., STECKA E. Calcium effect on number, dimension and activity of nucleoli in cortex cells of pea (*Pisum sativum* L.) roots after treatment with heavy metals. Plant Science **111,** 153, **1995.**
8. SIEDLECKA A., KRUPA Z. Cd/Fe interaction in higher plants – its consequences for the photosynthetic apparatus. Photosynthetica **36,** 321, **1999.**
9. MAŁKOWSKI E., STOLAREK J., KARCZ W. Toxic effect of Pb^{2+} ions on extension growth of cereal plants. Polish Journal of Environmental Studies **5** (1), 41, **1996**.
10. OBROUCHEVA N.V., BYSTROVA E.I., IVANOV V.B., ANTIPOVA O.V., SEREGIN I.V. Root growth responses to lead in young maize seedlings. Plant and Soil **200,** 55, **1998.**
11. KURTYKA R., MAŁKOWSKI E., KARCZ W., GALAS W., KITA A. Effect of calcium and cadmium on growth of maize seedlings and accumulation of both metals in their organs. Zeszyty Problemowe Postępów Nauk Rolniczych **488,** 697, **2002** [in Polish].

12. HASSETT J.J., MILLER J.E., KOEPPE D.E. Interaction of lead and cadmium on maize root growth and uptake of lead and cadmium by roots. Environmental Pollution **11**, 297, **1976**
13. JARVIS S.C., JONES L.H.P., HOPPER M.J. Cadmium uptake from solution by plants and its transport from roots to shoots. Plant and Soil **44**, 179, **1976**.
14. KHAN S., KHAN N.N. Influence of lead and cadmium on the growth and nutrient concentration of tomato (*Lycopersicum esculentum*) and egg-plant (*Solanum melongena*). Plant and Soil **74**, 387, **1983**.
15. TRIVEDI S., ERDEI L. Effects of cadmium and lead on the accumulation of Ca^{2+} and K^+ and on the influx and translocation of K^+ in wheat of low and high K^+ status. Physiologia Plantarum **84**, 94, **1992**.
16. SKÓRZYŃSKA-POLIT E., TUKENDORF A., SELSTAM E., BASZYŃSKI T. Calcium modifies Cd effect on runner bean plants. Environmental and Experimental Botany **40,** 275, **1998.**
17. HUANG J.W., CUNNINGHAM S.D. Lead phytoextraction: species variation in lead uptake and translocation. The New Phytologist **134,** 75, **1996**.
18. COX W.J., RAINS D.W. Effect of lime on lead uptake by five plant species. Journal of Environmental Quality **1**, 167, **1972.**
19. FLORIJN P.J., VAN BEUSICHEM M.L. Uptake and distribution of cadmium in maize inbred lines. Plant and Soil **150**, 25, **1993.**
20. FLORIJN P.J., NELEMANS J.A., VAN BEUSICHEM M.L. Evaluation of structural and physiological plant characteristics in relation to the distribution of cadmium in maize inbred lines. Plant and Soil **154**, 103, **1993.**
21. WALKER W.M., MILLER J.E., HASSETT J.J. Effect of lead and cadmium upon the calcium, magnesium, potassium, and phosphorus concentration in young corn plants. Soil Science **124**, 145, **1977**.
22. LAGRIFFOUL A., MOCQUOT B., MENCH M., VANGRONSVELD J. Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.). Plant and Soil **200**, 241, **1998**.
23. WIERZBICKA M., OBIDZIŃSKA J. The effect of lead on seed imbibition and germination in different plant species. Plant Science **137**, 155, **1998.**
24. GREGER M., LINDBERG S. Effects of Cd^{2+} and EDTA on young sugar beets (*Beta vulgaris*). II. Net uptake and distribution of Mg^{2+}, Ca^{2+} and Fe^{2+}/Fe^{3+}. Physiologia Plantarum **69**, 81, **1987**.
25. GREGER M., BERTELL G. Effects of Ca^{2+} and Cd^{2+} on the carbohydrate metabolism in sugar beet (*Beta vulgaris*). Journal of Experimental Botany **43**, 167, **1992.**
26. RUBIO M.I., ESCRIG I., MARTINEZ-CORTINA C., LÓPEZ-BENET F.J., SANZ A. Cadmium and nickel accumulation in rice plants. Effects on mineral nutrition and possible interactions of abscisic and gibberellic acids. Plant Growth Regulation **14**, 151, **1994.**
27. MAŁKOWSKI E., KITA A., GALAS W., KARCZ W., KUPERBERG J.M. Lead distribution in corn seedlings (*Zea mays* L.) and its effect on growth and the concentrations of potassium and calcium. Plant Growth Regulation **37,** 69, **2002**.
28. KIM Y.-Y., YANG Y.-Y., LEE Y. Pb and Cd uptake in rice roots. Physiologia Plantarum **116**, 368, **2002**.
29. CLEMENS S., ANTOSIEWICZ D.M., WARD J.M., SCHACHTMAN D.P., SCHROEDER J.I. The plant cDNA LCT1 mediates the uptake of calcium and cadmium in yeast. Proceedings of the National Academy of Science USA **95**, 12043, **1998**.
30. KARCZ W., STOLAREK J., LEKACZ H., KURTYKA R., BURDACH Z. Comparative investigation of auxin and fusicoccin-induced growth and H^+-extrusion in coleoptile segments of *Zea mays* L. Acta Physiologiae Plantarum **17**, 3, **1995**.
31. KARCZ W., BURDACH Z. A comparison of the effects of IAA and 4-Cl-IAA on growth, proton secretion and membrane potential in maize coleoptile segments. Journal of Experimental Botany **53**, 1089, **2002**.
32. MARSCHNER H. Mineral nutrition in higher plants. Academic Press Ltd., London, Greate Britain, pp. 889, **1995**.
33. ROOT R.A., MILLER R.J., KOEPPE D.E. Uptake of cadmium – its toxicity, and effect on the iron ratio in hydroponically grown corn. Journal of Environmental Quality **4,** 473, **1975.**
34. KELTJENS W.G., VAN BEUSICHEM M.L. Phytochelatins as biomarkers for heavy metal stress in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.): combined effects of copper and cadmium. Plant and Soil **203,** 119, **1998.**
35. BRENNAN M.A., SHELLEY M.L. A model of the uptake, translocation, and accumulation of lead (Pb) by maize for the purpose of phytoextraction. Ecological Engineering **12**, 271, **1999.**
36. WIERZBICKA M. Lead in the apoplast of *Allium cepa* L. root tips - ultrastructural studies. Plant Science **133**, 105, **1998**.
37. PRZYMUSIŃSKI R., WOŹNY A. The reactions of lupin roots on the presence of lead in the medium. Biochemie und Physiologie der Pflanzen **180**, 309, **1985**.
38. TUNG G., TEMPLE P.J. Uptake and localization of lead in corn (*Zea mays* L.) seedlings, a study by histochemical and electron microscopy. The Science of the Total Environment **188**, 71, **1996**.