

The effect of EDTA and EDDS on lead uptake and localization in hydroponically grown *Pisum sativum* L.

Sława Glińska · Sylwia Michlewska · Magdalena Gapińska · Piotr Seliger · Rafał Bartosiewicz

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Abstract *Pisum sativum* plants were treated for 3 days with an aqueous solution of 100 μM $\text{Pb}(\text{NO}_3)_2$ or with a mixture of lead nitrate and ethylenediaminetetraacetic acid (EDTA) or [S,S]-ethylenediaminedisuccinic acid (EDDS) at equimolar concentrations. Lead decline from the incubation media and its accumulation and localization at the morphological and ultrastructural levels as well as plant growth parameters (root growth, root and shoot dry weight) were estimated after 1 and 3 days of treatment. The tested chelators, especially EDTA, significantly diminished Pb uptake by plants as compared to the lead nitrate-treated material. Simultaneously, EDTA significantly enhanced Pb translocation from roots to shoots. In the presence of both chelates, plant growth parameters remained considerably higher than in the case of uncomplexed Pb. Considerable differences between the tested chelators were visible in Pb localization both at the morphological and ultrastructural level. In Pb+EDTA-treated roots, lead was mainly located in the apical parts, while in Pb+EDDS-exposed material Pb was evenly distributed along the whole root length.

Transmission electron microscopy and EDS analysis revealed that in meristematic cells of the roots incubated in Pb+EDTA, large electron-dense lead deposits were located in vacuoles and small granules were rarely noticed in cell walls or cytoplasm, while after Pb+EDDS treatment metal deposits were restricted to the border between plasma-lemma and cell wall. Such results imply different ways of transport of those complexed Pb forms.

Keywords EDDS · EDTA · Lead localization · Root meristem · Phytoextraction

Introduction

Numerous anthropogenic activities lead to an accelerated release of various heavy metals including Pb into the environment. Lead is one of the most dangerous pollutants, due to its long-time persistence (Mühlbachová 2011). It not only affects plant growth and productivity, but also enters the food chain causing hazards to man and animals (Zaier et al. 2010; Uzu et al. 2011). The possible negative impact of Pb on the environment and human health creates the need for remediation of contaminated areas. Phytoremediation has been proposed as an environmentally friendly and cost-effective alternative to conventional remediation technique (Seth et al. 2012; Singh et al. 2012).

Unfortunately, there are two limitations concerning Pb phytoremediation: its extremely low solubility in soils and its poor mobility (Andra et al. 2011). To enhance both the bio-availability of Pb and translocation from roots to harvestable parts of plants, synthetic chelators such as ethylenediaminetetraacetic acid (EDTA) and [S,S]-ethylenediaminedisuccinic acid (EDDS) have been proposed (Banaaraghi et al. 2010; Zhao et al. 2010; Gunawardana et al. 2011). EDTA has a very

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S. Glińska (✉) · S. Michlewska · M. Gapińska
Laboratory of Electron Microscopy, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland
e-mail: slawa@biol.uni.lodz.pl

P. Seliger
Department of Inorganic and Analytical Chemistry, University of Lodz, Tamka 12, 91-403 Lodz, Poland

R. Bartosiewicz
Laboratory of Electron Microscopy, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland

high chelate binding constant with Pb (log $K = 18.0$) (Niinae et al. 2008). The main drawback of EDTA is high persistence in the environment that can cause metal leaching into groundwater (Saifullah et al. 2009) and decrease in soil microbial activity (Mühlbachová 2011). Contrary to EDTA, EDDS has been shown to be easily biodegradable (7–30 days), to cause much smaller leaching of the metal into the soil profile and to be less toxic to soil microorganisms (Zhao et al. 2010; Mühlbachová 2011; Komárek et al. 2010). However, EDDS forms a weaker complex with Pb (log $K = 12.7$) (Niinae et al. 2008).

Phytoremediation involves three subsequent stages: (1) solubilization of metals in soil and their transfer to the root surface, (2) uptake into the roots, (3) translocation to the shoots. Most studies have focused on the first stage which is relatively well understood (Luo et al. 2006a; Lešťan et al. 2008; Lo et al. 2011); however, there is no clear evidence how chelated metal is taken up and distributed in plants (Luo et al. 2006b; Lešťan et al. 2008). Hydroponic experiments can be used to investigate these two processes to dispel doubts concerning the form of Pb absorption (chelated or ionic) and the route of transport.

The aim of this study was to compare the influence of EDTA and EDDS on lead absorption, translocation and localization in *Pisum sativum* seedlings grown in a hydroponic culture.

Materials and methods

Plant material and treatment

The seeds of *P. sativum* L. cv. Hówiecki were surface sterilized with 10 % sodium hypochlorite for 10 min and then rinsed extensively with distilled water. After soaking for 12 h in running water, they were placed on moistened filter in Petri dishes to germinate in the darkness at 22 °C. Two-day-old seedlings were transferred to aerated nutrient solution of the following composition: KNO_3 0.51 g/L, $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$ 1.18 g/L, $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ 1.23 g/L, H_2PO_4 0.14 g/L, Fe^{3+} 5 mg/L, with pH 6.0. The plants were grown under controlled conditions: light intensity of 170 $\mu\text{E}/\text{m}^2/\text{s}$ photoperiod 16/8 h and temperature 24 °C for 4 days. The growth medium was changed every 48 h. After that time, 35 plants were treated with 400 mL of aqueous solution of 100 μM $\text{Pb}(\text{NO}_3)_2$ or lead nitrate with EDTA or EDDS at equimolar concentrations for 3 days. Such conditions were chosen on the basis of Vassil et al. (1998) experiments. It was found that 1:1 molar ratio of Pb and EDTA optimized Pb–EDTA solubility. Plants cultured in distilled water were the control. The solutions were changed every 24 h. The experiment was repeated four times.

Pb content in the incubation medium

To check the changes in Pb content in each of the experimental variants as well as the form of Pb in Pb+EDTA and Pb+EDDS solutions (only chelated one or also ionic), the incubation media were analyzed before starting the experiment (0 day), as well as after the 1st and 3rd day of plant incubation. Pb content was calculated on the basis of the external standard addition method from the absorption spectra taken on the UV–VIS spectrophotometer.

Aqueous solutions of 5 mM $\text{Pb}(\text{NO}_3)_2$ (POCh), 5 mM EDTA (Aldrich) and 5 mM EDDS (Fluka) were prepared on the triple distilled water and used as standard solutions. All the reagents used were of analytical grade. Moreover, 1 mM standard complex solutions (Pb+EDTA and Pb+EDDS) were made from the above-mentioned standard solutions.

Due to the possible influence of the matrix effect on the absorption values of the incubation solutions, the standard addition method was used. Before measurement, all the investigated incubation media were centrifuged (1,006 g) to remove solid plant wastes. To minimize the influence of the matrix effect the reference solution was always taken from the control plant culture.

The following samples were prepared for spectrophotometric analysis:

For Pb^{2+} :

- Sample 1: 2 mL H_2O + 48 mL incubation medium
- Sample 2: 1 mL EDTA + 1 mL H_2O + 48 mL incubation medium
- Sample 3: 1 mL EDTA + 1 mL standard complex solution Pb+EDTA + 48 mL incubation medium
- Sample 4: 2 mL EDTA + 48 mL incubation medium

For complexes (Pb+chelator):

- Sample 1: 2 mL H_2O + 48 mL incubation medium
- Sample 2: 1 mL chelator + 1 mL H_2O + 48 mL incubation medium
- Sample 3: 1 mL standard complex solution (Pb+chelator) + 1 mL H_2O + 48 mL incubation medium
- Sample 4: 1 mL Pb^{2+} + 1 mL H_2O + 48 mL incubation medium

The absorbance of the solutions was measured on UV–VIS V-630 spectrophotometer (JASCO, Japan) equipped with quartz cuvettes at 230.0 and 241.4 nm for EDDS and EDTA, respectively, due to the maximum absorbance of the investigated complexes (Welcher 1958; Säbel et al. 2010).

Plant growth analysis

Root growth was determined after 1 and 3 days of incubation by subtracting the length of roots before incubation

from that after incubation. Shoot and root dry weight (DW) was estimated on the same day after drying the material for 2 days at 60 °C.

Lead uptake

To determine lead content in roots and shoots, 0.2 mg of dried plant material (washed in deionized water before drying) was digested with a mixture of 6.5 mL of concentrated nitric acid and 1 mL of 30 % H₂O₂ in a closed system at 200 °C in a microwave oven Ethos-1 (Milestone, Italy) for 40 min. The concentration of Pb was measured spectrophotometrically using ICP-OES OPTIMA 2000 DV (Perkin-Elmer, USA). Calibration was made using a multi-element standard (Merck).

In addition to the total metal content, both a bioaccumulation factor (BF) and a translocation factor (TF) were calculated. BF is defined as the ratio of metal concentration in the plant to that in the incubation medium and TF as the ratio of metal concentration in the shoots to that in the roots.

Lead localization

For lead localization at the morphological level, five seedlings from each experimental variant were placed in a 0.2 % solution of sodium rhodizonate (C₆Na₂O₆) in 0.1 M citrate buffer, pH 5.0 for 24 h, at 4 °C (Glińska and Gabara 2002). After repeated washing in distilled water, the seedlings were dried and their color was estimated. Brown-red color indicated the presence of lead. Photographic documentation was made using Power Shot A 640 digital camera (Canon).

For lead localization at the ultrastructural level, 2-mm-long root tips of 1-day-treated material (five for each variant) were fixed in 2 % glutaraldehyde in 0.1 M cacodylate buffer pH 7.2, for 2 h at 4 °C. Subsequently, they were rinsed with the same buffer and postfixed in 1 % osmium tetroxide for 2 h at 4 °C. The material was dehydrated in a graded ethanol series and embedded in Epon-Spur's resin mixture. Unstained ultrathin sections were examined in transmission electron microscope (TEM) JEM 1400 (JEOL Co., Japan, 2008) equipped with energy-dispersive full range X-ray microanalysis system (EDS INCA Energy TEM, Oxford Instruments, Great Britain) and high-resolution digital camera (CCD MORADA, SiS-Olympus, Germany).

Statistical analysis

Data are shown as means with the standard error (SE). The significance of differences between treatments was

determined by the Student's *t* test. Differences at $\alpha \leq 0.05$ were considered to be statistically significant.

Results

Pb content in the incubation solutions

The concentration of Pb in the Pb(NO₃)₂ solution drastically decreased after the first day of plant growth (Fig. 1). On the 3rd day, the amount of ions absorbed by plants from the medium was much lower and their content was only about 40 % of the initial concentration. In the case of Pb+chelator variants, the depletion of Pb concentration in the incubation media was much lower. Pb was least absorbed when it was given with EDTA and a slight drop which was noted on the 1st and the 3rd day was not statistically significant. In the case of Pb+EDDS variant, the Pb absorption was higher but almost tenfold lower than that in the case of Pb(NO₃)₂ solution.

The spectrophotometrical analysis of the incubation medium of Pb+EDTA and Pb+EDDS variants on 0 day was done to check whether Pb²⁺ ions were completely or partly bound by chelators. The obtained results showed that addition of the chelator standard solutions (EDTA or EDDS) to the samples (dash lines) did not cause any increase in absorption curve (as compared to the solid line of the incubation medium curve) (Fig. 2). It indicates that lead ions were completely chelated by both tested chelators and there was even an excess of EDDS (see the line after addition of Pb(NO₃)₂ standard solution as compared to the original sample).

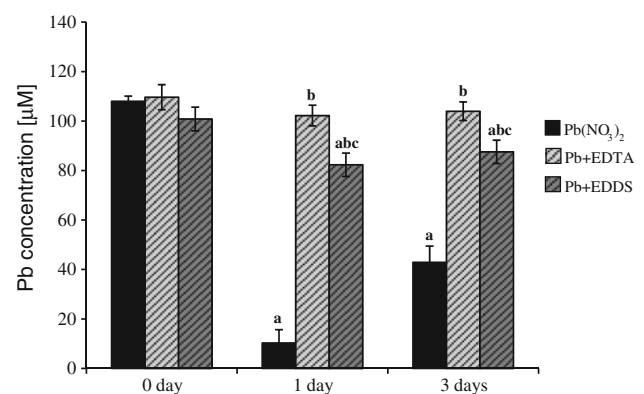
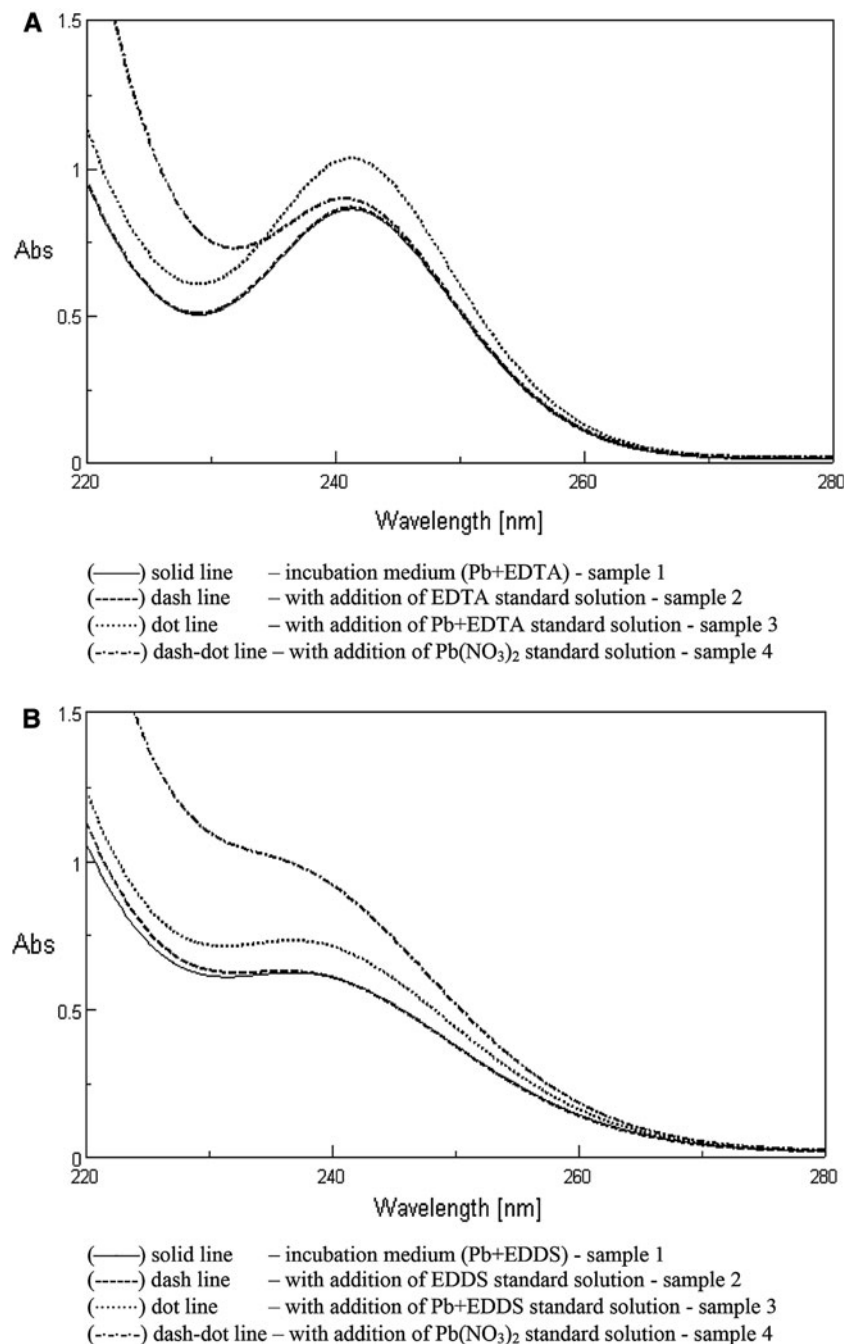


Fig. 1 Depletion of Pb concentration in the incubation media after 1 and 3 days of experiment with hydroponically growing *Pisum sativum* seedlings. Letters denote statistically significant differences between: ^atime 0 and 1st or 3rd day after treatment, ^bPb+chelator- and Pb(NO₃)₂-treated material within the same day of treatment, ^cboth chelator treatments on the same day of treatment. Student's *t* test distribution $\alpha \leq 0.05$

Fig. 2 Spectra of spectrophotometric analysis of the incubation media of Pb+EDTA (a) and Pb+EDDS (b) variants on the 0 day of experiment (before plant treatment)



Growth parameters

The presence of lead ions caused a 90 % drop in *P. sativum* root growth as compared to the control already after 1 day of incubation (Fig. 3). Similar reduction (96 %) persisted also after longer treatment. Neither tested form of Pb chelates affected root growth during the first day of experiment. However, prolonged root exposure to Pb+EDTA and Pb+EDDS brought about 44 and 35 % reduction in their growth, respectively, as compared to the control plants. Nevertheless, in the

presence of both chelates root growth remained considerably higher than in the case of uncomplexed Pb (Fig. 3).

The presence of Pb(NO₃)₂ reduced the root DW after 1 day of experiment by 15 %, while the mixture of EDTA or EDDS with lead nitrate did not cause any changes in this parameter as compared to the control (Fig. 3). After 3 days of culture in the presence of Pb²⁺ drop in the root DW was more significant—39 %. Pb+EDTA and Pb+EDDS reduced the root biomass less than Pb²⁺, by 31 and only 14 %, respectively (Fig. 3).

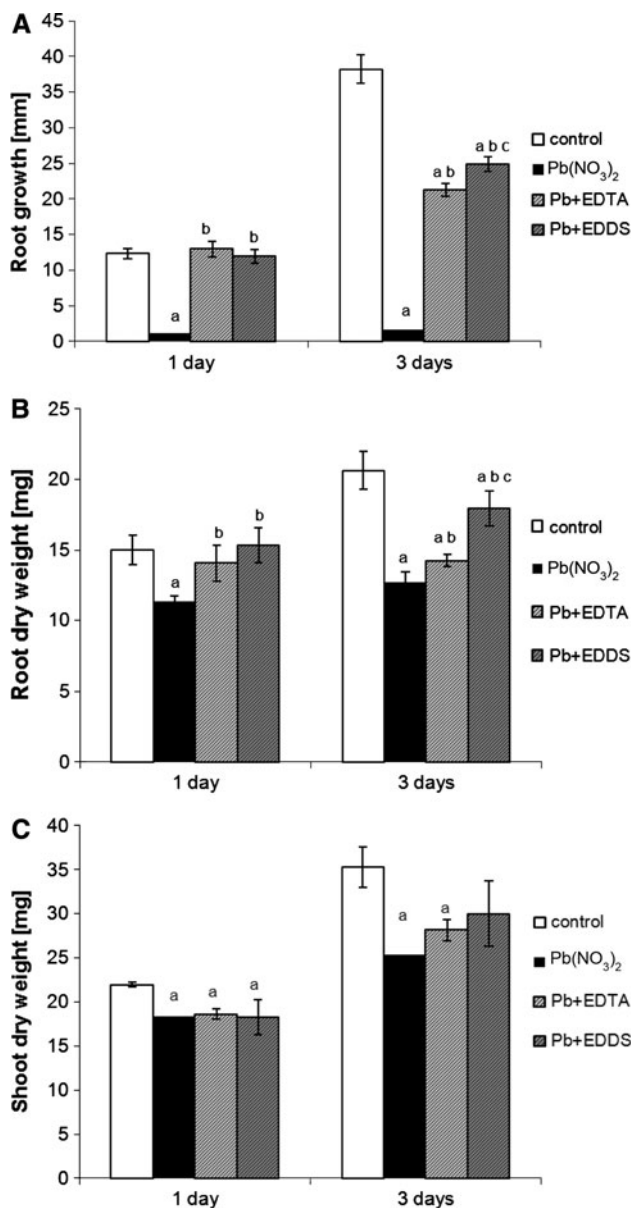


Fig. 3 Effect of EDTA and EDDS addition to the $\text{Pb}(\text{NO}_3)_2$ incubation medium on the growth parameters: root growth (a), root dry weight (b) and shoot dry weight (c) of *Pisum sativum* seedlings after 1 and 3 days of treatment. Letters denote statistically significant differences between: ^atreatment and control, ^bPb+chelator- and $\text{Pb}(\text{NO}_3)_2$ -treated material, ^cboth chelator treatments. Student's *t* test distribution $\alpha \leq 0.05$

After 1-day treatment the shoot DW was reduced by about 16 % in all experimental variants as compared to the control. Longer incubation in $\text{Pb}(\text{NO}_3)_2$ and Pb+EDTA resulted in more significant drop of shoot dry weight (by 28 and 20 % respectively). The shoot DW of plants treated with Pb+EDDS was not statistically different from the control (Fig. 3).

Lead uptake

The control seedlings contained only trace Pb amounts both in roots and shoots. In contrast, the seedlings growing for 1 day in the presence of $\text{Pb}(\text{NO}_3)_2$ accumulated 59 mg Pb/kg DW in shoots and as much as 10,110 mg Pb/kg DW in roots and after 3 days those values were much higher, 119 and 36,335 mg Pb/kg DW, respectively (Table 1). Root BF of Pb from lead nitrate solution was high already after short incubation (488.4) and at the end of experiment reached 1,755.3 (Table 2). Shoot BF was significantly lower, 2.9 and 5.7, respectively (Table 2). TF on both days was below 0.01 (Table 2).

The roots of plants treated with both examined Pb chelates accumulated considerably less Pb than those incubated in $\text{Pb}(\text{NO}_3)_2$. The Pb concentrations in the roots incubated in Pb+EDTA solution were 121 and 808 mg Pb/kg DW after 1 and 3 days of treatment, while those in Pb+EDDS were 218 and 5,304 mg Pb/kg DW, respectively (Table 1). The root BF was significantly higher in the presence of EDDS than EDTA, especially after 3 days of experiment (Table 2).

Both examined chelators, but especially EDTA, enhanced Pb translocation from roots to shoots (Table 2). However, the concentration of metal in the aboveground parts of plants was slightly lower than in the material treated only with lead (Table 1). TF decreased during the experiment, most remarkably in the case of Pb+EDDS (Table 2).

Lead localization

After 1 day of the experiment, sodium rhodizonate staining revealed the presence of Pb in the material growing in all three tested lead solutions. Only roots of the control plants were not stained. The main roots of lead nitrate-treated plants were intensively colored except for their basal parts (Fig. 4). The roots growing in Pb+EDTA and Pb+EDDS solutions were significantly less stained and differed in terms of metal localization. Pb+EDTA-treated material was characterized by Pb localization mainly in meristematic zones of main and lateral roots, while in Pb+EDDS-treated roots Pb was more evenly distributed along the meristem and elongation zone (Fig. 4).

After 3 days of incubation, the roots of $\text{Pb}(\text{NO}_3)_2$ -treated material were intensively red–brown stained along all their length (Fig. 4). The roots incubated in the mixture of Pb and EDTA or EDDS contained significantly less metal than those treated only with lead (Fig. 4). The roots of plants treated with Pb+EDTA were markedly stained in meristematic and elongation zones. The Pb+EDDS-treated roots were evenly stained along all their length (Fig. 4).

Table 1 Effect of EDTA and EDDS added to 100 μ M lead nitrate solution at equimolar ratio on the concentration of lead (mg/kg DW) in the root and shoot of *Pisum sativum* seedlings after 1 and 3 days of treatment

Treatment	Pb concentration (mg/kg DW)				
	1 day		3 days		
	Root	Shoot	Root	Shoot	
Control	11 \pm 1	3 \pm 1	12 \pm 1	2 \pm 1	
Pb(NO ₃) ₂	10,110 \pm 147a	59 \pm 4a	36,335 \pm 260a	119 \pm 3a	
Pb+EDTA	121 \pm 2ab	44 \pm 1ab	808 \pm 45ab	104 \pm 3ab	
Pb+EDDS	218 \pm 2abc	15 \pm 1abc	5,304 \pm 154abc	60 \pm 2abc	

Data are mean \pm SE of three replicates

Student's *t* test distribution $\alpha \leq 0.05$

Letters denote statistically significant differences between: ^atreatment and control, ^bPb+chelator- and Pb(NO₃)₂-treated material, ^cboth chelators treatments

Table 2 Effect of EDTA and EDDS added to 100 μ M lead nitrate solution at equimolar ratio on the bioaccumulation factors (BF) and translocation factor (TF) of Pb in *Pisum sativum* seedlings after 1 and 3 days of treatment

Treatment	1 day			3 days		
	BF _{root}	BF _{shoot}	TF	BF _{root}	BF _{shoot}	TF
Pb(NO ₃) ₂	488.4	2.9	0.006	1,755.3	5.7	0.003
Pb+EDTA	5.9	2.1	0.364	39.0	5.0	0.129
Pb+EDDS	10.5	0.7	0.069	256.2	2.9	0.011

Transmission electron microscopy revealed the presence of electron-dense black deposits in meristematic cells of *P. sativum* roots treated with Pb(NO₃)₂ as well as with Pb+EDTA or Pb+EDDS (Fig. 5). X-ray microanalysis confirmed the presence of lead in those structures, but not in similar gray deposits observed in vacuoles of the control material (Fig. 5a). Interestingly, the subcellular localization of Pb differed depending on the heavy metal form in the incubation solution. In the meristematic cells of Pb(NO₃)₂-treated roots, numerous small grains and bigger granules of Pb were located in cell walls and rather large metal deposits were observed in vacuoles (Fig. 5b). In Pb+EDTA-treated material large electron-dense lead deposits were located in vacuoles and small granules were rarely noticed in cell walls or cytoplasm (Fig. 5c). The localization of lead in meristematic cells of Pb+EDDS-treated roots was restricted to the electron-dense oval structures on the border between plasmalemma and a cell wall (Fig. 5d).

Discussion

Reduction of plant growth caused by lead was described for many species, both in soil (Cheyns et al. 2012; Shu

et al. 2012) and in hydroponic experiments (Piechalak et al. 2008; Zhivotovsky et al. 2011; Azad et al. 2011; Seth et al. 2011). Our results correspond with the earlier reports. We found that the decrease in dry weight of *P. sativum* shoots and roots was correlated with dramatic reduction of root elongation in the presence of ionic lead in the hydroponic solution. The presence of both tested chelators completely alleviated the toxic effect of lead on *P. sativum* root growth parameters in short-time exposure and significantly improved them after 3-day incubation. EDDS appeared to be slightly more effective. The mitigation of adverse effects of lead by EDTA was described earlier in hydroponically grown *P. sativum* (Piechalak et al. 2003), *Vicia faba* (Shahid et al. 2011) and also in *Sedum alfredii* (Tian et al. 2011). Ruley et al. (2006) evaluated the effects of chelators on the growth of *Sesbania drummondii* in soil contaminated with Pb(NO₃)₂. Plant shoot and root weights in the presence of Pb and EDTA were significantly higher than those in the presence of Pb alone. Also in hydroponically grown *Helianthus annuus*, Pb+EDTA resulted in lower toxicity as compared to ionic Pb (Seth et al. 2011). At equimolar concentrations of Pb and EDTA, formation of 100 % Pb–EDTA complex in the nutrient solution was observed that could result in alleviation of the toxicity both of free Pb and free EDTA (Saifullah et al. 2009; Tian et al. 2011). Spectrophotometric analysis of the incubation medium containing 100 μ M of Pb(NO₃)₂ and 100 μ M of EDTA or EDDS revealed that all Pb was in a chelated form and there was even excess of EDDS.

Two different phenomena may account for the limitation of Pb phytotoxic effect by synthetic chelators: (1) reduction of Pb uptake and (2) binding and stabilization of metal ions by the chelator which prevents Pb reaction with cell components. In our hydroponic experiment, both EDTA and EDDS significantly reduced absorption of Pb by *P. sativum* plants. Simultaneously, loss of Pb+EDDS complex from the incubation solution was tenfold lower than



Fig. 4 Morphological localization of lead with the use of rhodizonic method in *Pisum sativum* roots treated with aqueous solution of 100 μM $\text{Pb}(\text{NO}_3)_2$ or lead nitrate with EDTA or EDDS at equimolar concentrations for 1 (a) and 3 days (b)

loss of Pb^{2+} , while loss of $\text{Pb}+\text{EDTA}$ was even smaller. The same phenomenon was observed in hydroponically grown *H. annuus*: the $\text{Pb}+\text{EDDS}$ -treated plants had lower root metal concentration and no toxicity symptoms as compared to Pb -treated plants. However, shoot Pb uptake was significantly higher (22 times) in the case of $\text{Pb}+\text{EDDS}$ treatment as compared to $\text{Pb}(\text{NO}_3)_2$ solution (Tandy et al. 2006).

Synthetic chelators are used to remediate heavy metal-contaminated soils to enhance both metal availability and its translocation from root to shoot (Saifullah et al. 2009; Vamerali et al. 2010).

Both EDTA and EDDS addition to Pb -contaminated soils significantly increased metal uptake and its transport to the aboveground parts of plants (Wang et al. 2009; Kumar et al. 2011). However, EDTA was much more efficient than EDDS at enhancing root Pb uptake and its root-to-shoot translocation (Epelde et al. 2008).

In our experiment, despite the fact that the total metal uptake decreased, the translocation of Pb to the aboveground parts of *P. sativum* plants was significantly higher

in the presence of the tested chelators, especially EDTA. However, there are contradictory results concerning the effect of chelators on the total amount of the metal taken up from a hydroponic solution. The enhanced accumulation of Pb by hydroponically grown *Zea mays* (Wu et al. 1999), *P. sativum* (Piechalak et al. 2003) and *H. annuus* (Seth et al. 2011) was reported after EDTA addition to $\text{Pb}(\text{NO}_3)_2$ -containing nutrient solution. On the other hand, many authors observed decrease in Pb plant uptake in the presence of synthetic chelators in nutrient solutions (Tandy et al. 2006; Xu et al. 2007; Tian et al. 2011). Piechalak et al. (2008) demonstrated that the decrease in Pb uptake after application of the chelator was much evident at lower metal/chelator concentrations (27-fold at 0.1 mM as compared to 1.7-fold at 1.0 mM concentration). The above correlation implicates that $\text{Pb}+\text{EDTA}$ complex is not easily taken up by plants and its accumulation increases at high concentrations, after destruction of natural barriers.

Chelator complexes with metals are probably taken up along an apoplastic pathway (Tandy et al. 2006; Zhao et al. 2010). However, during the translocation from roots to

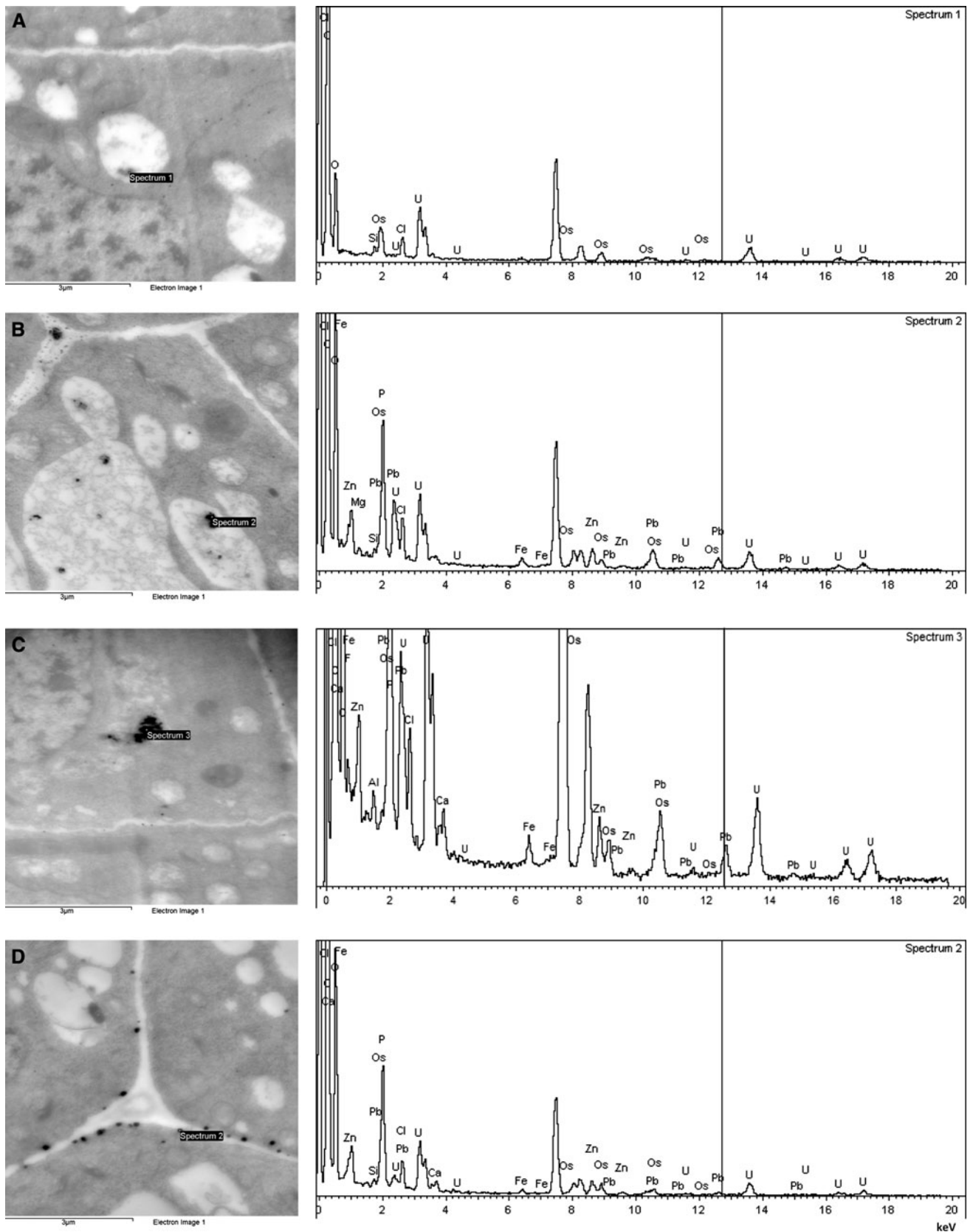


Fig. 5 Ultrastructural localization of lead by TEM analysis in meristematic cells of *Pisum sativum* roots treated for 1 day with distilled water—control (a), aqueous solution of 100 μM $\text{Pb}(\text{NO}_3)_2$

(b) and lead nitrate with EDTA (c) or EDDS (d) at equimolar concentrations with X-ray spectra (point analyses) from electron-dense deposits

shoots they meet the Casparin strip that halts apoplastic flow and forces them to cross cell membranes of endodermis. The physiological basis of the metal–chelator complex uptake and particularly the mechanisms allowing this negatively charged large molecule to cross the membrane are unknown. However, the Casparin strip is not a perfect barrier. At root tips it is not fully formed, and at the site where lateral roots protrude from the main root the Casparin strip can be disrupted. Niu et al. (2011) demonstrated in the hydroponic culture of *Z. mays* that at low concentrations the Cu–EDDS complex (200 μM) was passively absorbed mainly from the apoplastic spaces where lateral roots penetrate the endodermis. At higher concentrations (3,000 μM), the passage cells which form a physiological barrier controlling ion absorption were injured and substantially larger quantities of this complex could enter the root xylem. Moreover, it is suggested that chelators, mainly EDTA, could damage the membrane of root cells by chelating Zn^{2+} and Ca^{2+} cations that stabilize it (Vassil et al. 1998).

The rhodizonate method applied in our study revealed Pb presence in Pb+EDTA-treated plants mainly in the apical parts of roots where the endodermis barrier is not formed and both ways of transport are possible. As revealed by TEM and EDS, the electron-dense Pb deposits in Pb+EDTA-treated material were predominately located in vacuoles, and small granules were rarely noticed in cell walls or cytoplasm. Such Pb localization implicates both ways of transport of Pb taken up from Pb+EDTA solution. Jarvis and Leung (2001) came to the same conclusion after observing Pb deposits in cell walls, plasmodesmata and chloroplasts of Pb+EDTA-treated *Chamaecytisus proliferus* shoot parenchyma cells. Also, Zheng et al. (2012) suggested that Pb was transported both along apoplastic and symplastic pathways, independently of the presence or absence of EDTA. However, Sarret et al. (2001) revealed that the mixture of PbEDTA^{2-} and unidentified Pb species was present in the leaves of *Phaseolus vulgaris* grown in Pb+EDTA solution. Thus, the highly stable Pb–EDTA complex present in the solution can be partly dissociated when absorbed by a plant (Sarret et al. 2001).

The translocation factor of Pb in *P. sativum* plants growing in Pb+EDTA solution was 5- and 12-fold higher than that in plants incubated in Pb+EDDS after 1 and 3 days, respectively. It could be explained by the fact that Pb+EDDS complex is weaker and in the roots the cation exchange sites in cell walls competed with EDDS for Pb and split the complex (Tandy et al. 2006), so more Pb was bound to these sites and less was transported to the shoots. The even distribution of Pb in Pb+EDDS-treated *P. sativum* roots revealed by rhodizonate staining also seems to confirm such an explanation of lower metal translocation to shoots. Also, the ultrastructural localization of Pb

deposits in Pb+EDDS-treated *P. sativum* root on the border between the cell wall and plasmalemma could support this hypothesis and indicate apoplastic transport of Pb+EDDS.

Conclusions

In the presence of EDTA or EDDS, *P. sativum* growth parameters remained considerably higher than in the case of uncomplexed Pb, as metal absorption from the incubation media and its concentration in plants were significantly lower in the former case. The obtained results indicate that EDTA reduced Pb uptake by pea seedlings more efficiently than EDDS, but markedly stimulated the translocation of the metal from roots to shoots. The examined chelators differently affected Pb localization in the root meristem cells that implied different ways of transport of those complexed Pb forms.

Author contribution S. Glińska designed the experiment, collected and analyzed the data and wrote the manuscript. S. Michlewska prepared plant material for TEM and drafted figures. M. Gapińska participated in plant growth analysis. P. Seliger is responsible for measurements of Pb content in incubation media. R. Bartosiewicz helped X-ray microanalysis.

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Conflict of interest The authors declare that they have no conflict of interest.

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