

The Influence of Selenium on Root Growth and Oxidative Stress Induced by Lead in *Vicia faba* L. *minor* Plants

Magdalena Mroczek-Zdyrska · Małgorzata Wójcik

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Abstract The effect of selenium (Se) on *Vicia faba* L. *minor* roots subjected to lead (Pb) stress was studied by investigating root growth, root viability, and antioxidant enzyme activity. The experiments were carried out on plants grown for 2 weeks on Hoagland medium supplied with 50 μM Pb in the form of lead nitrate $\text{Pb}(\text{NO}_3)_2$ and/or Se concentrations of 1.5 and 6 μM in the form of sodium selenite Na_2SeO_3 . It was shown that Pb reduced the root growth and caused serious damage in the roots, which was accompanied by metal accumulation in these tissues. The exposition of roots to Pb led to significant changes in the biochemical parameters: the MDA and T-SH content and glutathione peroxidase (GSH-Px) activity increased but the guaiacol peroxidase (GPOX) activity decreased. Moreover, Pb intensified $\text{O}_2^{\bullet-}$ production in the roots. Selenium at a lower concentration alleviated Pb toxicity which was accompanied by a decreased $\text{O}_2^{\bullet-}$ production in the apical parts of roots and increased the T-SH content and GPOX activity. However, higher Se concentration intensified MDA and T-SH accumulation and GPOX and GSH-Px activity in Pb-treated plant roots. At low concentration, Se improved cell viability whereas at high concentration it was pro-oxidant and enhanced the lipid peroxidation and cell membrane injury.

Keywords Antioxidant enzymes · Lead · Oxidative stress · Selenium · *Vicia faba* L.

Abbreviations

CAT	Catalase
DHE	Dihydroethidium
DTNB	5,5'-Dithiobis(2-nitrobenzoic acid)
FDA	Fluorescein diacetate
GPOX	Guaiacol peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
MDA	Malondialdehyde
PI	Propidium iodide
SSA	Sulfosalicylic acid
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid

Introduction

Lead (Pb) is one of the most common and dangerous environmental contaminants [1]. Lead accumulation in plant tissues results in numerous disturbances of physiological processes. The most harmful effects of Pb phytotoxicity include alteration in the cell membrane permeability resulting in dehydration and water stress, inhibition of cell division, and a decrease in electron transport activities during photosynthesis and respiration [2, 3]. Lead binds strongly to sulfhydryl groups of proteins so its toxicity can be also attributed to distortion of enzymes and structural proteins [1]. It also causes visible symptoms of plant injury such as growth inhibition, chlorosis, or root tip browning [4].

M. Mroczek-Zdyrska (✉)
Department of Cell Biology, Maria Curie-Skłodowska University,
Akademicka 19,
20-033 Lublin, Poland
e-mail: magmr@interia.pl

M. Wójcik
Department of Plant Physiology,
Maria Curie-Skłodowska University,
Akademicka 19,
20-033 Lublin, Poland

The plant response to Pb contamination is a key research problem, and a special effort is undertaken in seeking factors which affect the reduction of Pb absorption or toxicity in plants. Selenium (Se) is one of the potential antagonists to Pb. Studies on animals have shown that Se limits the toxic effects of heavy metals and interacts with Pb [5], Cr [6], and Hg or Cd [7]. Recent publications indicate that Se addition may also alter the total content of tissue heavy metals by reducing their uptake by plants [8–11]. Selenium addition restrained accumulation of Pb and Cd in lettuce and enhanced absorption of some nutritional elements (Fe, Mn, Cu, Ca, and Mg) [8]. In rape (*Brassica napus*) seedlings, Se was found to reverse the Cd-induced decrease in fresh mass and changes in lipid unsaturation and peroxidation as well as changes in the DNA methylation pattern [12]. Also in broccoli (*Brassica oleracea*) plants, Se supplementation helped the plant to minimize the Cd toxicity by γ -tocopherol increases [10].

The effect of Se on plant organisms is not well recognized and its role in the plant metabolism is still controversial. Selenium is not an essential element for plants but it is taken up and metabolized in different ways depending on the plant species and its accumulation in tissues [13–15]. High Se concentrations are toxic to plants [16]. Selenium toxicity results from its incorporation into amino acids to substitute sulfur. Seleno-amino acids incorporated into protein cause replacement of S–S bonds by the less stable Se–Se bonds. In this way, the biological activity of the protein can be changed [13–15]. Moreover, high Se doses were found to generate superoxide radicals and enhance lipid peroxidation. On the contrary, low Se concentrations may have antioxidant properties and a positive impact on growth and development of plants [12, 16–20]. The antioxidative effect of Se may be attributed to the Se-induced increase in GSH-Px activity and APX activity and to an increased foliar concentration of antioxidant compounds such as ascorbate (AsA) and glutathione (GSH) [21]. Importantly, the boundary between the essential and toxic Se levels is relatively small and depends on many additional factors such as plant age, organs, genotype, or vegetation conditions [22].

The response of plants to Pb and Se interactions has been described by only a few authors [8, 9, 23, 24]. Exposure of plants to Pb may result in oxidative stress as indicated by lipid peroxidation and ROS production in tissues [25]. The ability of plants to overcome the effect of Pb stress may be related to the effective scavenging of oxygen species, such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH^\bullet), and superoxide radical ($\text{O}_2^{\bullet-}$). Both enzymatic and non-enzymatic antioxidants are involved in this process. Catalases and peroxidases are two major systems for the enzymatic removal of H_2O_2 in plants [26]. The non-enzymatic antioxidants may include GSH, AsA, and carotenoids.

The aim of the present study was to investigate the effect of Pb (lead nitrate) in combination with Se (sodium selenite)

on root growth and viability and on selected biochemical parameters (MDA and T-SH content) and antioxidant enzyme activity (CAT, GPOX, and GSH-Px). Moreover, the ability of Se to alleviate Pb toxicity in *Vicia faba* L. *minor* roots was tested and discussed.

Materials and Methods

Plant Material and Growth Conditions

Seeds of *Vicia faba* L. *minor* cv. Nadwiślański (field bean) germinated in the dark at 25°C for 7 days. Next, the seedlings were cultivated in 3-dm³ pots (10 plants per one pot) with modified full-strength Hoagland's medium [27] containing the following mineral components: KNO_3 (6 mM), $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (4 mM), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (2 mM), iron citrate (0.85 μM), H_3BO_3 (46 μM), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (9 μM), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.76 μM), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.32 μM), and $\text{H}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.11 μM). $\text{NH}_4\text{H}_2\text{PO}_4$ was removed from Hoagland's medium to prevent precipitation of lead phosphate [28]. To determine the effect of Pb and Se, the growth medium was supplemented with 50 μM Pb in the form of $\text{Pb}(\text{NO}_3)_2$ (Sigma) and/or 1.5 μM or 6 μM Se in the form of Na_2SeO_3 (Sigma). The Pb and Se concentrations were chosen on the basis of preliminary experiments and literature data. Two-week cultivation was performed at 25/20°C (day/night) with 16 h/8 h (day/night) photoperiod at photosynthetic active radiation 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$. During the experiment, the nutrient solution was continuously aerated and its losses were supplemented daily with dH_2O . The medium was changed once a week and its pH was kept at 5.0–5.2.

Analyses were performed on fresh material or material frozen in liquid nitrogen 14 days after Pb and/or Se addition, and the measurements were performed in triplicate. For the analysis of dry weight, the roots were dried at 70°C.

Determination of Pb and Se Content

Root and shoot samples of 0.25 g dry weight were mineralized in 10 ml HNO_3 using the microwave digestion system “MARS 5” (Varian). After digestion, the residues were diluted to 25 ml with Milli-Q water. Lead and selenium contents were analyzed by the AAS (atomic absorption spectrometry) method with acetylene flame atomization in the air (FAAS) with the SPEKTR AA spectrometer (Varian). Calibration curves were made using Pb and Se standard aqueous solutions “suprapur” pure (Merck).

Root Viability

Root viability was tested using FDA/PI mixture staining (FDA—12.5 $\mu\text{g ml}^{-1}$ and PI—5 mg ml^{-1}) [29]. Primary

root apical fragments (about 2 cm length) were gently incubated in FDA/PI mixture for a few minutes and then thoroughly rinsed with dH₂O. Observations were carried out in a drop of dH₂O under a fluorescent microscope Nikon Labophot 2A with excitation at 450–490 nm and emission at 520 nm.

Cell Death Measurement

Root cell death was estimated spectrophotometrically according to the method described by Tamás et al. [30]. The whole root systems were cut off, rinsed under tap water and next three times in dH₂O and stained in 0.25% Evans blue for 15 min. After staining, the roots were rinsed in dH₂O until complete removal of excess dye. Then 1.5-g root samples were homogenized in a mortar with 6 ml DMSO (dimethylsulfoxide). After homogenization, the plant material was centrifuged twice at 10,000×g for 15 min. The supernatant absorbance was read at 600 nm with the UV–VIS spectrophotometer (Helios Gamma).

Detection of Superoxide Anion (O₂^{•-})

Apical fragments of primary roots (about 2 cm length) were placed into a freshly prepared 10 μM DHE in 100 μM CaCl₂, pH=4.75 for 12 h in the dark at room temperature [31]. After staining, the roots were rinsed with dH₂O. Observations were carried out in a drop of dH₂O under a fluorescence microscope Nikon Labophot 2A with excitation at 450–490 nm and emission at 520 nm.

Estimation of Lipid Peroxidation

The level of lipid peroxidation was determined as 2-thiobarbituric acid (TBA) reactive metabolites—malondialdehyde (MDA) according to Heath and Packer [32]. Frozen root samples (0.5 g) were extracted in 5 ml of 0.5% TBA in 20% TCA with 250 μl BHT (butylated hydroxytoluene). The mixture was heated in a water bath at 95°C for 30 min, and after cooling it was centrifuged twice at 15,000×g at 4°C for 15 min. The specific absorbance (at 532 nm) of the extract and the non-specific background absorbance (at 600 nm) were measured with the UV–VIS spectrophotometer (Helios Gamma). The MDA content was calculated by the extinction coefficient (155 mmol⁻¹ cm⁻¹).

Determination of T-SH Content

The total content of SH groups was determined spectrophotometrically according to Maas et al. [33] with Ellman's reagent (DTNB). The frozen roots were homogenized in a cooled mortar in an extraction solution containing 0.15% ascorbic acid, 2% SSA, and 0.037% Na₂EDTA

(ethylenediaminetetraacetic acid disodium salt) (5:1, volume/weight). The homogenate was centrifuged twice at 10,000×g for 15 min at 4°C. Then 0.5 ml of the supernatant was mixed with 0.5 ml 1 M K₂HPO₄–KH₂PO₄ buffer, pH=8.0, and 0.1 ml of 10 mM DTNB, and the absorbance was read at λ=412 nm after 1 min with the UV–VIS spectrophotometer (Helios Gamma). The SH group content was read from the standard curve prepared for L-cysteine.

Analyses of Antioxidant Enzyme Activity

Antioxidant enzyme activities were determined after they were extracted in 5 ml (CAT and GPOX) or 2.5 ml (GSH-Px) of 50 mM potassium phosphate buffer, pH=7.0, with 1 mM EDTA (ethylenediaminetetraacetic acid) and 1% PVPP (polyvinylpolypyrrolidone) and centrifuged twice at 10,000×g at 4°C for 15 min.

CAT (E.C. 1.11.1.6) activity was determined in the fresh plant material (1 g) according to Aebi [34]. Then 1.5 ml of 50 mM potassium phosphate buffer, pH=7.0, and 0.5 ml of 0.15% H₂O₂ were added to the quartz cuvette. The reaction was initiated by adding 5 μl of the supernatant to the reaction mixture at 25°C. The absorbance decrease (Δ Abs/min) was read at λ=240 nm for 30 s with the UV–VIS spectrophotometer (Helios Gamma). The calculation includes the molar absorption coefficient of H₂O₂ (ε=0.036 mM⁻¹ cm⁻¹).

GPOX (E.C. 1.11.1.7.) activity was determined in the frozen plant material (0.5 g) according to Velikova et al. [26]. The reaction mixture contained 2.75 ml of 50 mM phosphate buffer, pH=7.0, with 1% guaiacol and 100 μl of supernatant. The reaction was initiated by adding 150 μl 100 mM H₂O₂ to the reaction mixture and absorbance (Δ Abs/min) was read at λ=470 nm after 30 s with the UV–VIS spectrophotometer (Helios Gamma). The calculation includes the molar absorption coefficient of guaiacol (ε=26.6 mM⁻¹ cm⁻¹).

GSH-Px (E.C. 1.11.1.9.) activity was determined in the frozen plant material (0.5 g) according to Ali et al. [35]. The reaction mixture contained 50 mM potassium phosphate buffer, pH=7.0, 1 mM Na₂EDTA, 0.25 U ml⁻¹ GR (EC 1.6.4.2), 1 mM L-GSH, 0.15 mM β-NADPH, 1 mM NaN₃ (sodium azide), and the homogenate. The reaction was initiated by adding 1.5 mM t-BOOH (*tert*-butyl hydroperoxide) at 37°C and the absorbance decrease was measured with the UV–VIS spectrophotometer (Helios Gamma) at λ=340 nm. The reaction with t-BOOH allowed measurements of activity of GSH-Px containing selenium (without measuring the activity of GSH-Px containing no selenium). The unit of GSH-Px activity was an amount of enzyme that catalyzes the oxidation of β-NADPH (β-nicotinamide adenine dinucleotide phosphate reduced form) to β-NADP⁺ (β-nicotinamide adenine dinucleotide phosphate, oxidized form)

(mM min⁻¹ mg⁻¹ protein) at 25±2°C with the molar absorption coefficient for the β-NADPH (ε=6.22 mM⁻¹ cm⁻¹).

The Protein Content

The protein content was determined according to Bradford [36] using BSA (bovine serum albumin) as a standard with the UV–VIS spectrophotometer (Helios Gamma) 15 min after addition of color dye (Bio-Rad Protein Assay) at λ=595 nm±10 nm.

Statistical Analysis

The statistical analysis was performed using one-way ANOVA and Tukey's test at the significance level $p < 0.05$. Means and standard errors (SE) were used.

Results

Selenium and Pb Accumulation and Plant Growth

The selenium concentration in plant roots increased with increasing Se concentration in the growth medium (Table 1). Lead presence had no influence on the Se content in the roots. Also, addition of Se to the Pb-treated plants did not alter Pb accumulation in the roots.

The primary root elongation of the Pb-treated plants was significantly reduced (by 40%) in relation to the control plants (Table 2). There were no statistically significant differences in the primary root elongation in the Se-treated plants in comparison to the control. Also, addition of Se (1.5 μM or 6 μM) did not influence significantly the primary root elongation of the Pb-treated plants. In the 50 μM Pb-

Table 1 Lead and selenium contents in *Vicia faba* L. *minor* roots grown for 14 days in the control solution or in the presence of Pb and/or Se

Pb and/or Se concentration (μM)	(mg kg ⁻¹ DW)	
	Pb	Se
0	ND	388.00 a
1.5 Se	ND	614.00 b
6 Se	ND	844.75 c
50 Pb	13,630.33 a	371.61 a
50 Pb+1.5 Se	11,592.33 a	578.00 b
50 Pb+6 Se	12,981.33 a	926.00 c

Values are means, SE=8–40%. Values with different letters in the same column differ significantly from each other at $p < 0.05$. Limit detections: 6.2 (Pb) and 8.6 (Se)

ND not detectable

treated plants, the fresh weight of roots was reduced by 20% in relation to the control (Table 2). In the 1.5 μM Se-treated plants, a 34% increase in the root fresh weight was found; however, the fresh weight of roots in the 6 μM Se-supplied plants did not differ from the control. Addition of Se (both at 1.5 μM and 6 μM concentrations) to the Pb-treated plants did not change their fresh biomass production. The dry weight of roots was reduced only in plants treated with Pb and Se (by 19% and 29% at 1.5 and 6 μM Se, respectively) in comparison to the control (Table 2).

Root Viability

After incubation in FDA/PI mixture, the roots of the control plants showed an intense yellow-green fluorescence testifying to high cell viability (Fig. 1a). However, in the 50 μM Pb-treated roots, a strong reduction in cell viability was found, especially in the cap and dermatogen regions, as well as in parts more distant from the apex root—in the endoderm and the cylinder axis. In the 1.5 μM Se-treated plants, a slight decrease in cell viability was observed only in the apical parts of the roots, while at 6 μM Se, the cell mortality was more pronounced and was found in the apical meristem and on the root surface. In the 50 μM Pb+1.5 μM Se-treated roots, a slight reduction in cell viability was noted, mainly in the surface layer of the cells at the root apex. On the other hand, in the 50 μM Pb+6 μM Se-treated roots, a considerable increase in cell mortality in comparison to the roots exposed to Pb alone was observed, especially in the apical part of the roots. However, a group of living and green fluorescing cells in the apical meristem was still observed (Fig. 1a).

The observations of root viability were confirmed by quantitative analysis of root cell death (Fig. 1b). The presence of 50 μM Pb in the medium lowered the cell viability of roots by 83% in comparison to the control. In the roots of plants treated with 1.5 μM Se, the cell death was lower by 24%, whereas at 6 μM Se it was over two times higher in comparison to the control. The addition of 1.5 μM Se to the medium with 50 μM Pb improved root viability of Pb-treated plants by 45%; however, 6 μM Se drastically increased (by 84%) root cell mortality.

Oxidative Stress Analysis in Roots

Visualization of O₂^{•-} accumulation in plant roots is presented in Fig. 2. In the control plants, only weak fluorescence testifying to O₂^{•-} presence was observed in the apical root parts. In 1.5 μM Se-treated roots, O₂^{•-} accumulation was on the control level; however, it slightly increased at 6 μM Se. Pb intensified clearly the O₂^{•-} production. Strong, orange fluorescence was observed in the apical meristem

Table 2 The root growth of *Vicia faba* L. *minor* plants cultivated for 14 days in the control solution or in the presence of Pb and/or Se

Pb and/or Se concentration (μM)	Root elongation (cm)	Root fresh weight (mg plant^{-1})	Root dry weight (mg plant^{-1})
0	10.76 \pm 1.22 a	959.33 \pm 20.39 a	64.47 \pm 2.06 ab
1.5 Se	10.69 \pm 0.98 a	1290.00 \pm 67.58 b	74.04 \pm 3.88 a
6 Se	9.89 \pm 1.44 ab	938.67 \pm 31.06 a	63.08 \pm 3.30 abc
50 Pb	6.50 \pm 0.46 b	771.33 \pm 23.44 c	55.03 \pm 1.84 bcd
50 Pb+1.5 Se	5.97 \pm 0.92 b	736.00 \pm 30.92 c	52.40 \pm 3.09 cd
50 Pb+6 Se	7.59 \pm 0.51 ab	654.67 \pm 25.78 c	45.76 \pm 2.06 d

Values are means \pm SE ($n=15$). Values with different letters in the same column differ significantly from each other at $p<0.05$

and in the elongation zone of the roots of the Pb-treated plants both in the absence and presence of Se.

The increase in accumulation of lipid peroxides is indicative of enhanced production of reactive oxygen species. The level of MDA, one of the major TBA reactive metab-

olites, increased in the 50 μM Pb-treated roots by 39% in comparison to the control (Fig. 3). Also, 1.5 μM Se did not significantly influence the MDA level, while 6 μM Se increased the lipid peroxidation by 50% in comparison to the control. Moreover, while 1.5 μM Se did not have a

Fig. 1 Cell viability of *Vicia faba* L. *minor* roots after 14 days of cultivation in the control solution or in the presence of Pb and/or Se. **a** Visualization of root viability by FDA/PI staining. Yellow-green fluorescence indicates viable cells, red fluorescence indicates dead cells. **b** Quantitative analysis of root cell death after Evans blue staining. The bars indicate mean ($n=9$) \pm SE. Values with different letters differ significantly from each other at $p<0.05$

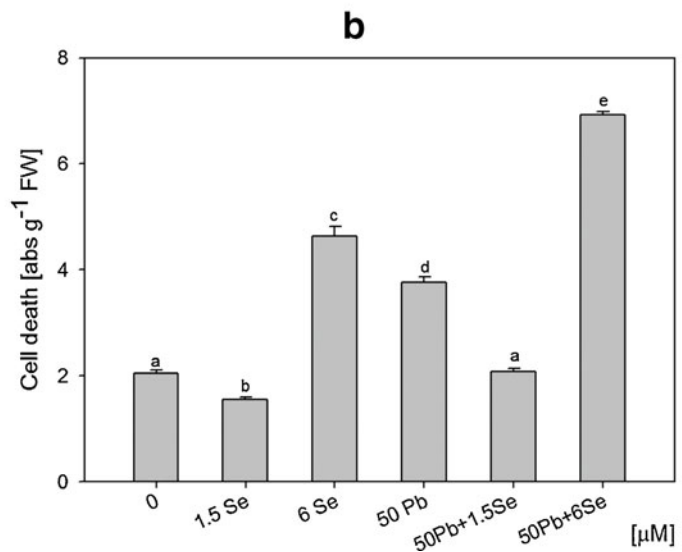
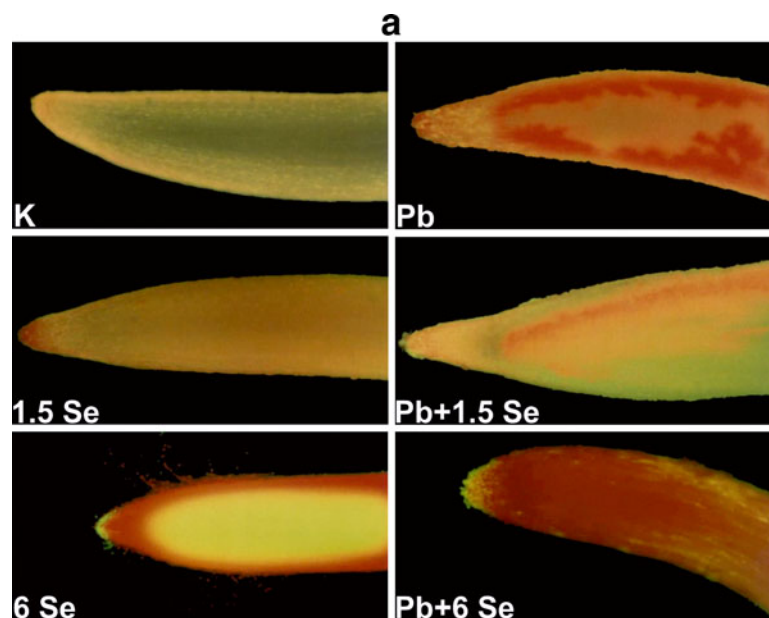
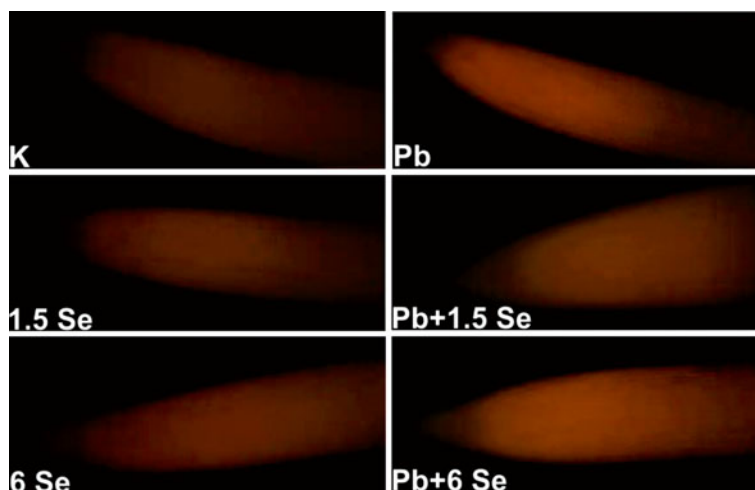


Fig. 2 Visualization of superoxide anion accumulation (after DHE staining) in *Vicia faba* L. *minor* roots after 14 days of cultivation in the control solution or in the presence of Pb and/or Se



significant effect on the MDA content in the roots of plants treated with Pb, 6 μM Se intensified lipid peroxidation in such plants by 18%. At 50 μM Pb+1.5 μM Se, the level of MDA was higher by 30% and at 50 μM Pb+6 μM Se by 64% than in the control roots.

Fifty micromolar of Pb significantly increased the total content of SH groups in the roots and the average concentration of T-SH was about 131% higher than in the control (Fig. 4). Similarly, Se increased the T-SH content, by 59% and 109% at 1.5 μM Se and 6 μM Se T-SH, respectively, in comparison to the control. The highest concentration of thiol compounds was found in the roots treated with Pb and Se (about three times higher than in the control). Selenium addition to the medium with Pb increased the thiol compounds synthesis by 35–42% in comparison to the plants treated with Pb alone.

At the 1.5 μM Se treatment, CAT activity decreased by 36% in relation to the control (Fig. 5a). Treatments with

6 μM Se as well as 50 μM Pb and 50 μM Pb+1.5 μM Se or Pb+6 μM Se did not change CAT activity.

In the roots treated with Pb alone or 1.5 μM and 6 μM Se alone, the GPOX activity was decreased by 15%, 12%, and 36%, respectively (Fig. 5b). In the roots exposed to 50 μM Pb+1.5 μM Se, the GPOX activity was at the control level, but after addition of 6 μM Se together with Pb, a 50% increase in GPOX activity was found in comparison to the control. Addition of Se, both 1.5 μM and 6 μM , to the Pb-treated plants increased GPOX activity by 10% and 77%, respectively, in comparison to plants supplemented with Pb alone.

Selenium did not influence the GSH-Px activity, whereas Pb induced a 60% increase in GSH-Px activity in comparison to the control (Fig. 5c). Addition of 1.5 μM Se to Pb-treated plants did not alter the GSH-Px activity in the roots while 6 μM Se increased the activity of this enzyme by 89%. The GSH-Px activity was higher in these plants by

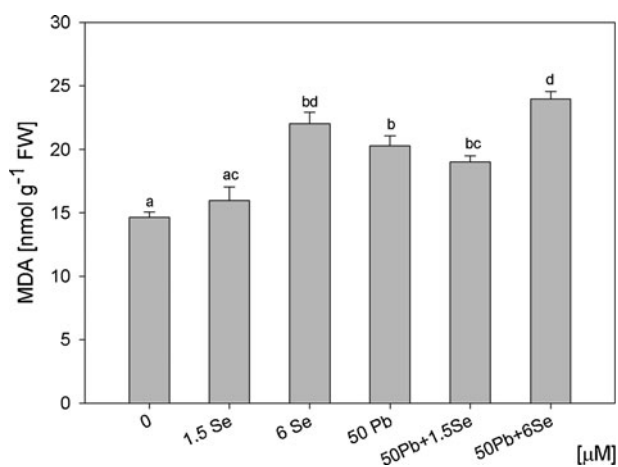


Fig. 3 Level of lipid peroxidation expressed in terms of MDA concentration in the roots of *Vicia faba* L. *minor* plants after 14 days of cultivation in the control solution or in the presence of Pb and/or Se. The bars indicate mean ($n=9$) \pm SE. Values with different letters differ significantly from each other at $p<0.05$

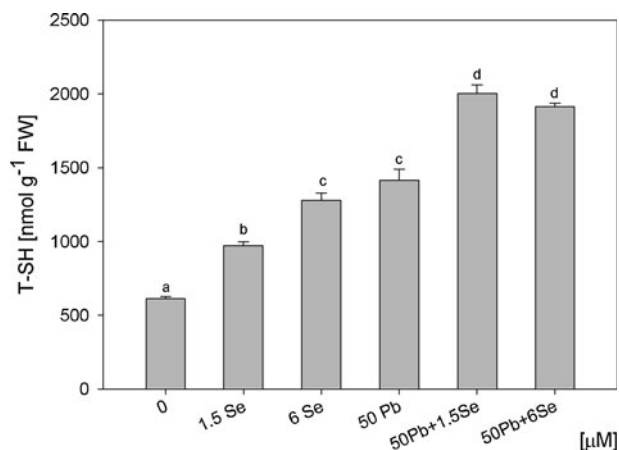


Fig. 4 Total SH content (T-SH) in the roots of *Vicia faba* L. *minor* plants after 14 days of cultivation in the control solution or in the presence of Pb and/or Se. The bars indicate mean ($n=9$) \pm SE. Values with different letters differ significantly from each other at $p<0.05$

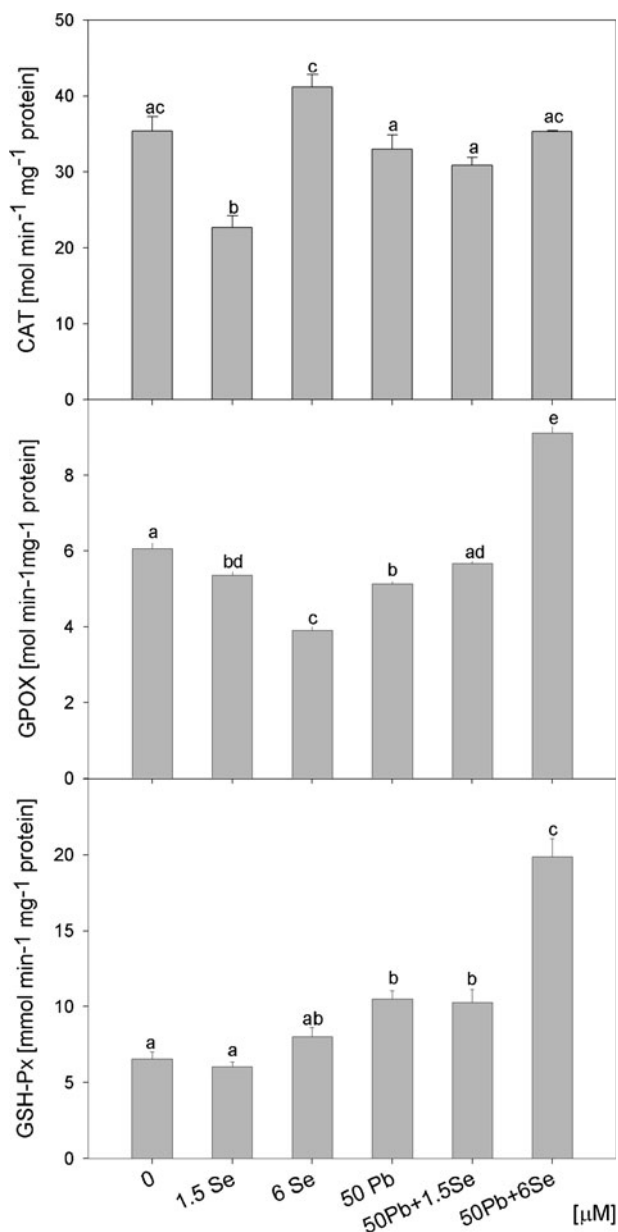


Fig. 5 Antioxidant enzyme activity in the roots of *Vicia faba L. minor* plants after 14 days of cultivation in the control solution or in the presence of Pb and/or Se. The bars indicate mean ($n=9$) \pm SE. Values with different letters differ significantly from each other at $p<0.05$

about 57% at 1.5 μ M Se and over two-fold at 6 μ M Se in comparison to the control.

Discussion

Root growth inhibition is a well-known and primary response of plants to Pb toxicity [4, 28, 37]. Our results revealed that roots were sensitive to toxic Pb ions. After 14 days of the experiment, both length and biomass of the root system of *Vicia faba L. minor* was significantly

reduced. It is believed that the decrease in fresh and dry weights is related to the reduction in the metabolic rate of cells and poor development of the root system may cause Pb-induced inhibition of water uptake [2]. However, that was not the case in this experiment since neither the dry weight nor water content (data not shown) was disturbed in the roots after the Pb treatment. The significant increase in fresh weight and water content (data not shown) in the 1.5 μ M Se-supplemented plants may be linked with the role Se plays in the regulation of water and an increase in tissue hydration. Selenium may contribute to more efficient water uptake by roots or reduce the intensity of transpiration and thereby reduce water loss in tissues [38].

The root growth inhibition induced by the Pb treatment was correlated with the internal Pb content within the tissues. It is well known that plant roots are the main organs which participate in Pb accumulation and that only small portions of Pb are translocated into shoots [4, 37]. The selenium content increased in the roots with the increasing concentration of Se in the nutrient solution. However, Se did not influence Pb accumulation in the roots. Our results are in agreement with the studies on rape, which indicated that Se (2 μ M) only insignificantly decreased Cd accumulation [12].

Cell death is the most visible symptom of damage caused by heavy metals. The plasmalemma is the first functional structure to come into contact with heavy metals [39]. Therefore, Pb accumulation in cells depends mainly on membrane permeability. A strong decrease in the viability of root cells was observed after Pb application. The lower Se concentration used increased and the higher Se concentration decreased the root viability of the Pb-treated plants. It was found that in the roots treated with 6 μ M Se alone or with Pb, necrotic cells were mainly localized in the surface rhizodermis layers and in the apical part of the roots. However, a group of living and metabolically active cells were observed in the apical meristem. This group of cells, which is characterized by an intense metabolism and identified as a quiet center (QC), is assumed to participate in the reconstruction of the apical meristem under stress conditions. The fact that the initial cells in plant roots under physiological conditions do not show a typical meristematic activity is commonly known. Their inactivity in “normal” top roots is due to the activity of other meristem parts [40]. The increased metabolic activity of QC cells, which was observed after the introduction of Se or Se + Pb to the growth medium, can testify to the initiation of the activity of this area by Se and its participation in the adaptation and tolerance of the roots to Pb.

There is a strong relationship between cell death and elevated ROS production. It is well known that Pb may contribute to generation of ROS and lead to oxidative stress in plant tissues [25]. Our results indicated the severe cell

death that in roots exposed to Pb was accompanied by an increase in $O_2^{\bullet-}$ production. Especially strong DHE fluorescence testifying to $O_2^{\bullet-}$ accumulation was observed in the apical meristem and in the endoderm—the main areas of cell death in the roots. Introduction of 1.5 μM Se to the medium with Pb decreased the intensity of DHE fluorescence in the roots.

Another indicator of cell membrane damage is lipid peroxidation. The increase in lipid peroxidation products modifies such membrane properties as flow, permeability, and enzyme activity. Many studies show that low Se concentrations reduce lipid peroxidation, whereas higher Se concentrations increase lipid peroxidation [16, 18, 19]. De la Luz Mora et al. [41] reported that the lowest level of lipid peroxidation in clover shoots was noted when the tissue Se concentration did not exceed 200 $\mu\text{g Se kg}^{-1}$ DW. Increased lipid peroxidation was found above this concentration. It seems that the significant increase in lipid peroxidation in the roots of our plants treated with 6 μM Se and 50 μM Pb+6 μM Se can be associated with the significant increase in the Se content.

Lead and other heavy metal ions often reduce cellular activities by generation of oxidative stress and inhibition of enzyme reactions. Oxidoreductase enzymes (CAT or GSH-Px) are the most sensitive enzymes to selenium due to the antioxidant properties of this element [42]. In our research, Pb did not influence CAT activity in roots. However, application of lower Se levels markedly decreased CAT. Literature data are ambiguous in regards to the effect of Se on CAT activity. Djanaguiraman et al. [19] observed that catalase did not participate in active H_2O_2 reduction. The reason for this could be that CAT is present only in the peroxisome and has low substrate affinity, as it requires simultaneous access of two molecules of H_2O_2 [43].

Exposition of plants to Pb stress decreased the GPOX activity by 15%. Similar results were obtained for Se. The results presented in our study indicate that Se increased the GPOX activity in roots exposed to Pb stress which may be indicative of its antioxidant properties. Numerous studies indicate that lower Se concentrations contribute to the increase in GPOX activity and thus have a beneficial effect on the antioxidant system in plants, while higher Se concentrations decrease the GPOX activity and may exhibit pro-oxidative properties [20, 41, 42].

According to the literature, the antioxidant properties of Se are connected with its beneficial effect on the GSH-Px activity [16, 17]. An increase in the GSH-Px activity was observed using either selenate or selenite [18]. However, in our studies no such Se effect on GSH-Px activity was found. Se did not change the activity of this enzyme, unlike Pb which significantly increased the GSH-Px activity both in absence and in presence of Se in the growth medium.

Two types of proteins rich in –SH groups are identified in plants that may be involved in binding of heavy metals:

phytochelatins (PCs) and metallothioneins (MTs) [44]. There are more numerous reports about induction of compounds rich in –SH groups under the influence of heavy metal rather than under non-metals such as selenium [45]. In a study on spinach and tomatoes, an increase in –SH groups after Se treatment was observed, but their content depended on the Se concentration and form [46]. In the present study, increased T-SH synthesis was observed after Pb and/or Se supplementation.

The protective role of Se on the Pb stress that we observed in the changes in root growth, viability, ROS production, and antioxidant activity is not unambiguous and depends on the Se concentration mainly. Selenium exerts dual effects on Pb toxicity: at a low concentration it improves the cell viability, whereas at a high concentration it is pro-oxidant, enhancing the accumulation of lipid peroxidation products and percent injury of the cell membrane.

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