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Accumulation and detoxification of lead ions in legumes

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Abstract

This study focuses on lead accumulation in roots, stems and leaves of three plant species of the Fabacea family: *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris* grown hydroponically in a medium supplemented with 1 mM concentration of lead. The largest amount of lead, up to 75 mg Pb/g dry weight, was accumulated in roots of *P. vulgaris*. The highest rate of Pb ions uptake from the medium took place during the first 10 h of incubation with lead and after 96 h of incubation lead content in the medium decreased by half. Thus, it was suggested that *P. vulgaris* could be used in rhizofiltration—the use of plant roots to absorb pollutants from water contaminated with lead. At the same time we studied the influence of lead on acid soluble thiol, glutathione, homoglutathione contents and the synthesis of phyto- and homophytochelatins in roots of *V. faba, P. sativum* and *P. vulgaris* grown hydroponically. Activation of the detoxicative-phytochelatin system was observed in the cytosol of root cells of the tested plants. This system was composed of phytochelatins (PCs) in roots of *V. faba,* homophytochelatins (hPCs) in *P. vulgaris* roots and both PCs and hPCs in *P. sativum* roots. The total content of PCs and hPCs in roots of *P. sativum* was very high and reached around 4800 (expressed in nmol $SH \times g^{-1}FW$) and induction of their synthesis occurred after only 2 h of treatment with 1 mM Pb. © 2002 Published by Elsevier Science Ltd.

Keywords: Phytoremediation; Phytochelatins; Homophytochelatins; Detoxification; Lead ions

1. Introduction

Plants usually show the ability to accumulate large amounts of lead without visible changes in their appearance or yield. In many plants, the level of lead accumulation can exceed even several hundred times the threshold of 2 mg/kg dry mass which is the maximum level permissible for man, without having any negative effect on their growth or yield (Wierzbicka, 1995). Therefore, it seems that plants can endure a level of environmental pollution that might be even several times higher than the level observed nowadays. The most polluted area in Poland is Silesia, where the content of lead in soils reaches 300–700 mg/kg dry mass (Kucharski et al., 1999). The most difficult problem connected with lead contamination of soil is its persistence. The present accumulation of lead in soil will remain there for about 300 years affecting to a lesser or greater degree plants grown on such sites. Thus, lead will be introduced into the food chain and biological circulation. Owing to their ability to accumulate lead and other heavy metals (HM) in tissues, plants can be used in the process of soil remediation and rhizofiltration.

There are well-known plants that are hyperaccumulators of metals, which can accumulate metals in their leaves and stems up to a concentration of 0.1% of dry mass (Baker, 1995). The hyperaccumulator plants can accumulate 1000 times more heavy metal than normal plants without any visible signs of toxicity. This group of plants includes about 400 species (Morel et al., 1997). There are 5 recognised plant species, which are hyperaccumulators of lead (Baker and Brooks, 1989), in which lead concentration exceeds 0.1% of dry wt; these are*: Armeria martima, Thlaspi rotundifolium*, *Thlaspi alpestre*, *Alyssum wulfenianum*, *Polycarpaea synandra*.

Kumar et al. (1995) showed that plants of the Brassicaceae family are good accumulators of lead, especially *Brassica juncea*. Indian mustard turned out to be the best lead accumulator as it can accumulate in its shoots about 10 mg Pb/g dry wt, and in roots even above 100 mg Pb/g dry wt after 20 days of cultivation in a substrate containing 625 μg Pb/g dry wt.

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The strategies of lead tolerance in plants are poorly understood. The tolerant ecotypes survive in an environment contaminated with lead and adopt mechanisms of avoidance and inactivation. Inactivation of lead, as well as of other heavy metals, is connected with the detoxification mechanism, which consists in the synthesis of thiol peptides-phytochelatins with the formula (γ-Glu-Cys)n- Gly, where $n = 2–11$, in plants, fungi, algae and marine diatoms. (Grill et al., 1989; Maitani et al., 1996; Rauser, 1995). These peptides are synthesised enzymatically from endogenically contained glutathione or its homologs due to the reaction catalyzed by phytochelatin synthase—an enzyme activated by heavy metal ions including Pb^{2+}.

γ-Glutamylocysteine dipeptidyl transpeptidase—(EC 2.3.2.15.) is a cytoplasmic constitutive enzyme. PC synthase genes were identified independently by three laboratories in *Saccharomyces pombe*, *Arabidopsis thaliana* and *Triticum aestivum* (Ha et al., 1999; Vatamaniuk et al., 1999; Clemens et al., 1999). Evidence of its activity was found in *Silene cucubalus* (Grill et al., 1989), in tomatoes (Chen et al., 1997), *Arabidopsis* (Howden et al., 1995; Vatamaniuk et al., 1999) and *Pisum sativum* (Klapheck et al., 1995). Owing to the high content of cysteine, phytochelatins—also refereed to as class III metallothioneins (Rauser, 1995; Maitani et al., 1996)—are able to create complex compounds with toxic ions of metals. These complexes transport HM into the vacuole by the ABC transporter which is localized in the tonoplast (Oritz et al., 1995), thus separating them from cell metabolism. A vacuolar HMT1 transporter (Cobbett, 2000; Li et al., 1997) catalyses MgATP-dependent uptake of both Cd^{2+}–PCs complexes and apo-PCs. The aim of this study was to examine three legumes of the Fabacea family: *Vicia faba, Phaseolus vulgaris* and *Pisum sativum* with the focus on lead accumulation in hydroponic cultures in order to establish if they could be used to clean water in rhizofiltration process.

The earlier research had already shown (Tomaszewska et al., 1996) that yellow lupin—a representative of the same group of plants—accumulates in its roots as much as 30 mg Pb/g dry wt, as well as the fact that lead ions induce, in its roots, the synthesis of phytochelatins from glutathione as the only substrate.

Lead tolerance strategies of these plants have not been fully explained yet. In our research, we have tried to explain the correlation between lead accumulation in different plant organs and the synthesis of phytochelatin complexing toxic lead ions in root cells and their transport from roots to above-ground plant organs.

2. Results

Our research materials were three legumes: *P. sativum*, *P. vulgaris* and *V. faba*. These plants were grown hydroponically in a medium supplemented with lead ions. The studied legumes showed high tolerance to large amounts of sublethal 10^{-3} M Pb in Hoagland medium. No visible changes were observed in the appearance of the above-ground parts of plants after 4 days of treatment with metal ions. In comparison to the control plants grown without Pb, the appearance of the roots of the Pb-exposed plants changed significantly. We observed a number of changes in the shape and, especially, in the appearance of roots in the plants treated with lead. The colour of the roots changed gradually under the influence of metal ions, from creamy white to dark brown, which was caused by intense suberification. The elongation growth of roots was slowed down and the number of hair roots decreased, which led to lowered water uptake. Index of tolerance (IT) was determined according to the Wilkins' method (Wilkins, 1957) for the three studied plants. The IT values of the examined plants were significantly different (Table 1) after 4 days of lead treatment. Differences between IT values indicate that *V. faba* has the highest resistance among the three plants and has the fastest initiation of the detoxicative system. Although *P. vulgaris* exhibits the highest sensitivity to lead ions (IT = 19%), it has the capacity to take up the largest amount of Pb (Fig. 1).

We found that roots were the main accumulation site of Pb in the plants we studied (Figs. 1 and 2), which is consistent with the earlier data concerning other plants. A steady growth in the level of the accumulated metal was observed until the end of the 96-hour period when the concentration of lead in roots of *P. vulgaris* reached 75 mg Pb/g dry wt. At the same time, roots of *P. sativum* and *V. faba* accumulated lead to a lesser degree (Fig. 1). After 96 h of cultivation with $Pb(NO_3)_2$,we found about 50 mg Pb/g dry wt in roots of *P. sativum,* and 46 mg Pb/g dry wt in roots of *V. faba*. The presented results showed that about 90–95% of the total amount of lead was localised in roots and only 5–10% was transported to the above-ground plant parts in all three plants after 96 h of lead treatment (Figs. 1 and 2). The level of lead in stems and leaves of all the studied plants increased with the time of lead treatment (Fig. 2). In stems the maximum level of Pb in *P. vulgaris* was 0.5 mg Pb/g fr. wt, in *P. sativum*—0.4 mg Pb/g fr. wt, and in

Table 1
Tolerance index—estimated by means of the Wilkins' test for *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris roots* grown hydroponically in Hoagland medium in the presence of 1 mM $Pb(NO_3)_2$

Time of lead treatment (h)	*Vicia faba* (%)	*Pisum sativum* (%)	*Phaseolus vulgaris* (%)
24	108.70	90.00	80.00
48	92.00	61.50	48.31
72	74.00	55.40	35.28
96	58.00	39.10	19.23

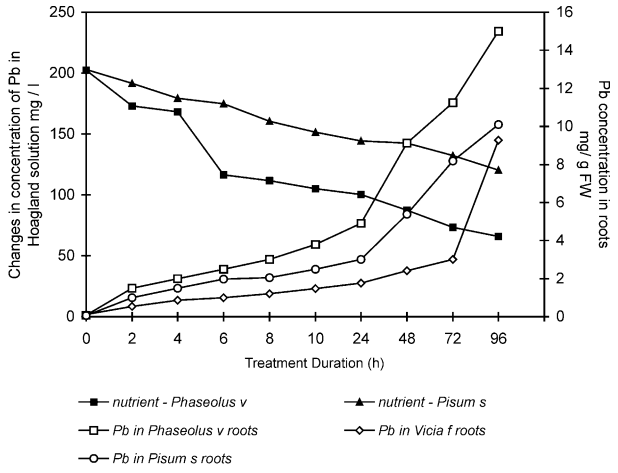

Fig. 1. The relation between changes in Pb content (mg/g FW.) in roots of *V. faba*, *P. vulgaris* and *Pisum sativum* grown hydroponically in Hoagland medium in the presence of 1 mM $Pb(NO_3)_2$ for 96 h and removal of Pb by 3-day-old seedlings of *P. vulgaris* and *P. sativum* from Hoagland medium during 96 h of lead treatment.

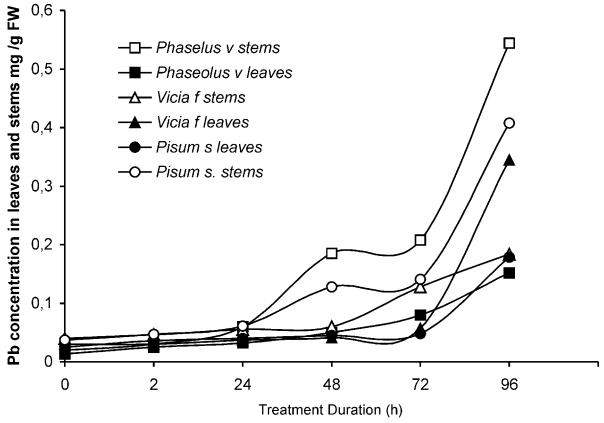

Fig. 2. Changes in Pb content (mg/g fr. wt) in stems and leaves of *V. faba*, *P. vulgaris* and *P. sativum* grown hydroponically in Hoagland medium in the presence of 1 mM $Pb(NO_3)_2$ for 96 h.

V. faba—less than 0.2 mg Pb/g fr. wt- the lowest value among the three plants. These values were, respectively, 30 times (*P. vulgaris*, *P. sativum*) and 50 times (*V. faba*) lower than the lead content in the roots. A significant increase in lead content was observed in leaves of *P. vulgaris*, *P. sativum* and *V. faba* after 72 h (Fig. 2). The highest amount of lead was found in leaves of *V. faba*—0.35 mg Pb/g fr. wt. Lower contents of accumulated Pb were found in leaves of bean and pea. The maximum levels in leaves of both plants did not exceed 0.2 mg Pb/g fr. wt. The next step in our research was monitoring changes of Pb concentration in the medium during four days of cultivation in the presence of lead ions (Fig. 1).

P. vulgaris showed the best capacity to absorb and take up lead ions from the nutrient medium. Roots of *P. vulgaris* lowered the lead concentration in the medium by half of the initial value within the first ten hours. Whereas, during the next 3 days of exposition, the rate of metal uptake was significantly slower and *P. vulgaris* roots absorbed only about 18% of the remaining Pb in the medium and after 96 h of treatment plants removed about 70% of Pb from the medium. *P. sativum* roots showed lower capacity of taking up Pb from the medium than *P. vulgaris*. The plants took up about 25% of the metal, i.e. almost a half less than removed by *P. vulgaris* during the first 10 h. Similarly as in the case of bean the rate of uptake was falling during a longer exposition time and in the time interval 24–96 h of cultivation pea roots took up only further 15% of Pb in the medium. Lead concentration in roots of the investigated plants in mg Pb/g fr. wt increased during 4 days of lead uptake from the medium (Fig. 1). Simultaneously, lead concentration in the Hoagland medium decreased. In stems and leaves, however, lead concentration did not increase significantly until the 72nd hour after lead ion treatment, and only then did a significant increase in the metal concentration take place (Fig. 2).

At the next stage of research, we studied the Pb content in the root compartments of the tested legumes. It was found that most of the lead content in roots is localised in the insoluble fraction of cell walls and nucleus, the so-called pellet (99% of lead in *P. vulgaris*, 97,5% in *P. sativum* and 95% in *V. faba*) (Fig. 3). We demonstrated that the highest amount of the accumulated metal—about 98%—was formed in the so-called pellet fraction, which is essentially the fraction of the cell walls and nucleus. Only 1% Pb/g fr. wt was in the roots of *P. vulgaris* treated with Pb^{+2} for 96 h (it was 0.15 mg Pb/g fr. wt) in a soluble fraction of root cells. Such concentration of Pb in the supernatant of root cells proved to be sufficient to activate phytochelatin synthase (EC.2.3.2.15) in *P. sativum*, *V. faba* and *P. vulgaris* to synthesise phytochelatins and homophytochelatins.

We found only glutathione (Fig. 4 A) in the roots of the control plants of *V. faba*, its homolog—homoglutathione (γGlu-Cys-βAla)—only in *P. vulgaris* (Fig. 4C), and both in *P. sativum* roots (Fig. 4B). Similar results were obtained by Klapheck (1988). The induction of the synthesis of glutathione—a substrate in the synthesis of phytochelatins—and its homologs, was observed in plants treated with Pb ions during the first 10 h of incubation. The level of GSH in *P. sativum* reached its highest value after 8 h (Kasierska et al., 2000). We observed a gradual decrease in the homoglutathione level during 96 h of incubation with Pb in roots of *P. vulgaris* (Table 2). The fastest responses to contamination with Pb ions were observed in *V. faba* root cells (Table 2; Fig. 5A, Fig. 6), in which the synthesis of phytochelatins took place after 2 h of lead treatment. The level of phytochelatins rose gradually

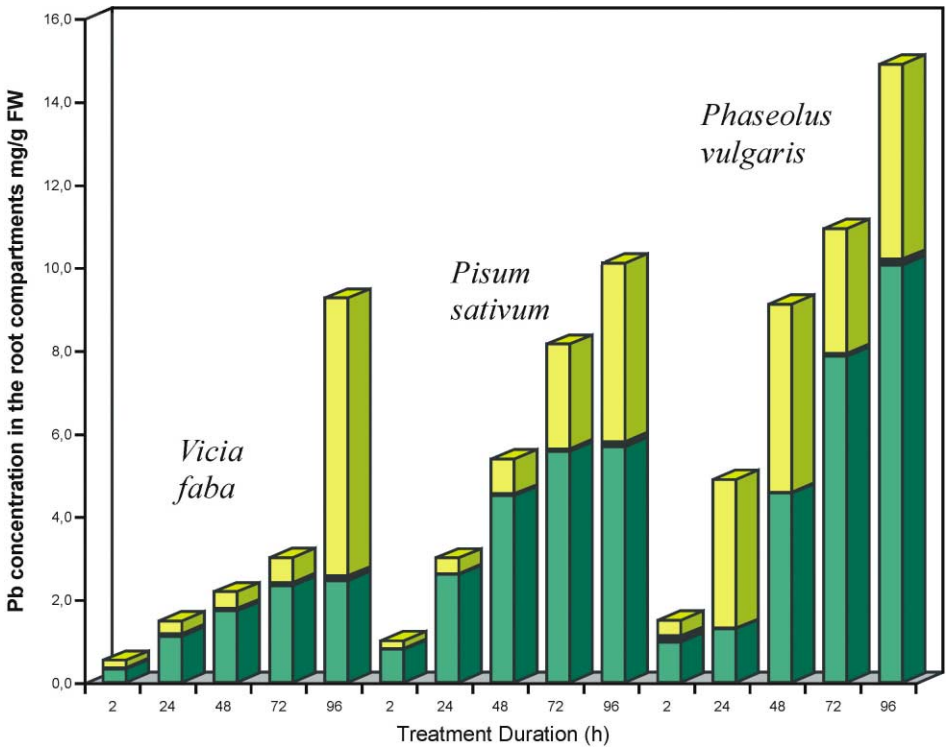

Fig. 3. Changes in Pb content (mg/g fr. wt) in roots (pellet , supernatant and surface) of *Vicia faba*, *Phaseolus vulgaris* and *Pisum sativum* grown hydroponically in Hoagland medium in the presence of 1 mM $Pb(NO_3)_2$ for 96 h.

reaching their maximum value after 8 h of Pb treatment (Table 2; Fig. 6). After a longer period of lead treatment a gradual decrease in the level of phytochelatins was observed until they disappeared completely. The synthesis of phytochelatins in the roots of *P. sativum* also took place after 2 h and their level rose reaching the maximum value after 72 h of incubation (Table 2; Figs. 5B, and 7). Homophytochelatins appeared in *P. sativum* after 24 h of lead treatment and their level increased gradually until the 72nd h. In the roots of *P. vulgaris,* there are only homophytochelatins which appear after 24 h of incubation with Pb and reach the maximum after 72 h (Table 2; Figs. 5C and 8).

3. Discussion

In examined plants we observed a few unfavourable changes in the appearance of the Pb-treated roots, for example browning, the lowered number of hair roots, growth inhibition, a decreased biomass growth.

An unfavourable effect of heavy metal ions, for example Pb, on roots was also observed by other researchers. For example, Wierzbicka (1995) observed that lead ions cause water deficit by disturbing water balance; she proves that it is one of the main factors which causes a poorer growth and development of plants in an environment contaminated with heavy metals. Geebelen et al. (1999) found a visible reduction of the root growth in *P. vulgaris* already at 80 μM Pb. This was correlated with a decrease in the root mass by as much as 90% at 400 μM $Pb(NO_3)_2$. Seregin and Ivanov (1998) also observed 50% inhibition of root growth in maize at 10^{-4} M lead nitrate and browning of roots treated with Pb ions. Gabara and Golaszewska (1992) observed changes in the appearance of roots correlated with a decrease in dry and fresh mass after lead treatment.

It is a well-known fact that roots provide the primary route for the penetration of heavy metal ions. Roots can take up 3–50 times more lead than leaves (Wozny et al., 1995). The plant we had studied earlier—yellow lupin- and pea, bean and broad bean which are examined here, accumulated up to 95% Pb in roots (Tomaszewska et al., 1996). Studying a number of species of the Brassicacea family and other plants Kumar et al. (1995) also demonstrated that about 90% of Pb accumulation occurred in roots. The amount of lead ions in the roots of the studied *P. vulgaris* is comparable with the results obtained by Burzynski (1987), who exposed *P. vulgaris,* cucumber and wheat to 10^{-3} M $PbCl_2$. He showed that cucumber is a plant which accumulates the largest amounts of Pb, but it was very sensitive to this concentration of $PbCl_2$ and, consequently, the plant was killed after

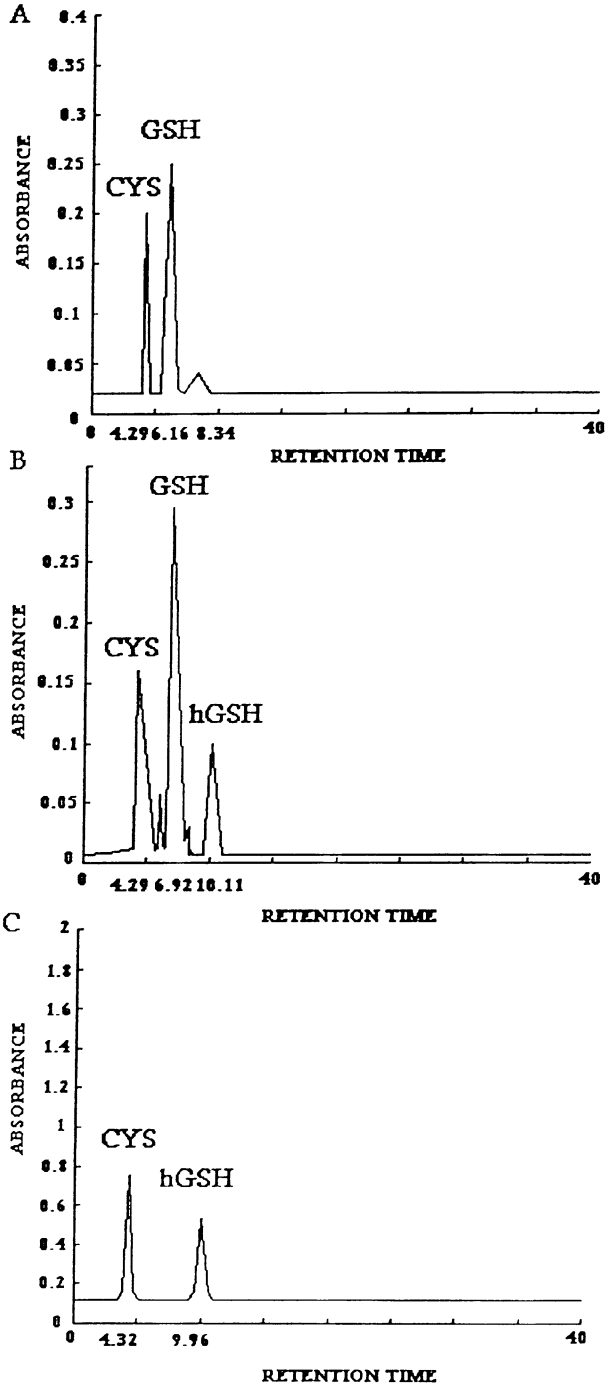

Fig. 4. HPLC chromatogram from root extract of legumes grown hydroponically in Hoagland medium for 72 h. (A) *Vicia faba*; (B) *Pisum sativum*; (C) *Phaseolus vulgaris*; Cys—cysteine, GSH—glutathione, hGSH—homoglutathione, a—unidentified thiols.

only 24 h of exposition. He also demonstrated that *P. vulgaris* accumulated over 75 mg Pb/g dry wt in roots and exhibited a significant tolerance to this metal (Burzynski, 1987). Antosiewicz and Wierzbicka (1999) localised the highest level of lead in cell walls of *Allium cepa* root tips by means of the conventional electron microscopy preparative technique. Seregin and Ivanov (1998) also obtained similar results in maize treated with 10^{-4} M $Pb(NO_3)_2$. This can be explained by the fact that lead was fixed by functional components of cell walls, such as, among others, polysaccharides (Seregin and Ivanov, 1998). Leopold and Gunther 1997 observed in *Silene vulgaris* cell cultures that only 5% of the total amount of metal ions was present in a soluble extract.

In our research, the investigated plants, i.e. *P. sativum* and *V. faba* accumulated much lower amounts of Pb than *P. vulgaris,* approximately 50 mg Pb/g dry wt. In comparison, the content of this metal in roots of *B. juncea*, which is thought to be a moderate accumulator of lead, was over 100 mg Pb/g dry wt and about 10 mg Pb/g dry wt in stems, that is, 10 times less than in roots (Kumar et al., 1995). The level of Pb in the roots of plants investigated by Kumar et al. (1995) ranged from 0.82 to 10.9% of dry wt, whereas in our investigations it was 7.5% of dry weight in *P. vulgaris*, and 5.1% of dry wt in *P. sativum*. The best accumulator among the studied plants of the Brassicacea family proved to be a variety of *B. juncea* (L) cultivar 426308 accumulating 188.3 mg Pb/g dry wt in roots and up to 34.5 mg Pb/g dry wt (3.5% of dry wt) in shoots.

The unique ability to accumulate lead, which is especially characteristic of *P. vulgaris,* could be exploited in rhizofiltration of lead from polluted water. One cannot exclude the fact that genetic manipulations might lead to creating a variety of *P. vulgaris,* which could have higher phytoextraction ability. Moreover, in the recent research on lead accumulation in *B. juncea,* it has been demonstrated that application of synthetic chelators, such as EDTA, both into soil (Epstein et al., 1999; Blaylock et al., 1997) and hydroponic cultures (Vassil et al., 1998; Salt et al., 1997) raised the concentration of soluble lead and increased its uptake by the plant by creating Pb-EDTA complexes. These results suggest that further studies should be conducted on the effect of this chelator on lead uptake by *P. vulgaris*. One should expect that application of EDTA into the hydroponic nutrient medium can possibly raise the degree of lead translocation into the above-ground parts of this plant.

Similarly, a significant increase in lead accumulation occurred in the roots of *B. juncea*, however, in the shoots of Indian mustard cultivated in a hydroponic medium (Kumar et al., 1995) it increased significantly only when lead ions concentration reached the value of 100 mg Pb/l in solution. Metals, which had been taken up by roots, were then loaded in the xylem sap and translocated to the above-ground parts of plants through the transpiration stream (Briat and Lebrun, 1999).

It is thought that some plants possess a mechanism which limits the translocation of metals to shoots (Antosiewicz, 1992). There are some unique plants—metallophytes—which are hyperaccumulators that accumulate a high amount of metals in shoots (Antosiewicz, 1992; Baker, 1995).

Table 2
The effect of lead on changes of glutathione, homoglutathione and phytochelatins, homophytochelatins contents (nmol SH × g^{-1} fr. wt) in roots of *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris* after 96 h 1 mM Pb $(NO_3)_2$ treatment

Treatment duration (h)	*Vicia faba*		*Pisum sativum*				*Phaseolus vulgaris*	
	GSH	PC	GSH	hGSH	PC	hPC	hGSH	hPC
0	97.2±9	ND	370±11	ND	ND	ND	287±34	ND
2	57±6	58.5±15	696±47	ND	46±9	ND	259±30	ND
8	45.9±8	83.6±22	1557±98	ND	182±37	ND	242±25	ND
24	50.8±5	34.9±13	729±45	69±15	1745±145	246±30	198±36	81±13
48	49.6±6	15.7±10	822±58	113±28	2727±270	574±42	159±26	178±25
72	30.3±7	ND	768±47	207±36	4547±320	368±33	121±22	298±32
96	21.5±6	ND	551±40	239±52	3958±299	312±37	67±19	60±15

GSH—glutathione, hGSH—homoglutathione, PC—phytochelatins, hPC—homophytochelatins, ND—not detected.
Data are means ±S.E. of three to four samples.

We demonstrated that the synthesis of thiol peptides, both phyto- and, homophytochelatins, takes place under the influence of Pb ions in root cells of the three tested legumes (Fig. 5). The high amounts of these peptides, which reached up to ca. 4500 nmol SH/ g FW, were formed in the roots of *P. sativum* (Table 2; Fig. 7), despite the fact that this plant had a medium tolerance index value, while the concentration of phytochelatins in the roots of *V. faba*, was much lower (Table 2; Fig. 6) but their induction took place after only 2 h. Therefore, one can assume that a higher IT of *V. faba* is not correlated with the amount of synthesised phytochelatins but rather with the rapid initiation of this cytosolic detoxication system which consists of phytochelatins and homophytochelatins, able to transport Pb–PCs and Pb–hPCs complexes through the cytosol into vacuoles. *P. vulgaris* showed the lowest IT value and medium quantitative amount of homophytochelatins (Table 2; Fig. 8) while it accumulated the most Pb.

The observed disappearance of phytochelatins in roots of *V. faba* may suggest that phytochelatins play only a transient role in the process of lead ions detoxification. Leopold et al. (1999) observed a similar effect in tomato and *S. vulgaris* cultures exposed to Cd^{2+} and Cu^{2+} ions (0.1 mM), where PC-metal complexes disappeared between the 7th and 14th day of treatment. According to these authors, this proves that the formation of phytochelatin-metal complexes plays a part only in the early stage of plant response to stress caused by heavy metal ions, and this is not related with an increased tolerance of plants to metals. Leopold and Gunther (1997) and Leopold et al. (1999) also analysed extracts of cell cultures and plants using the online coupled HPLC/ICP–MS system. They observed that eluants from HPLC column were complexes of Cd and Cu ions with ligands (molecular weight about 13 000 Da), which correspond to the phytochelatin molecule PC_2 with oxidised SH-groups. But in tomato and *S. vulgaris* cell cultures exposed to Pb and Zn ions, heavy metals were bound to a lower molecular weight ligands (they could not be sufficiently purified for mass spectrometry), but not phytochelatins. Mehra et al. (1995) also studied the binding of Pb to phytochelatins; they used UV/visible and circular dichroism (CD) spectroscopy. These authors suggest that Pb formed a complex with phytochelatins PC_2, PC_3 and PC_4. Both PC_2 and PC_3 appeared to bind one lead ion per peptide molecule. They suggest too that glutathione is probably the in vivo donor of heavy metals to PCs (potentially toxic heavy metal ions are first chelated by GSH and then transferred to PCs for eventual sequestration). Pb ions are then transferred from shorter chain PCs to longer chain ones which indicates that metal-binding strength increases with the chain length. Mehra and Mulchandani (1995) also present the role of GSH as an effective donor of heavy metal ions.

The relation between the level of PCs and the toxic metal effect was observed (Sneller et al., 1999) in roots of *S. vulgaris* treated with Cd^{2+} ions for 3 days. The linear relation between growth inhibition and concentration of PCs was noticed only at low Cd concentrations—1 and 2 μM $CdNO_3$, when growth inhibition was measured as time-dependent Effect Index. This correlation was still maintained at prolonged exposition to 1 and 2 μM Cd, whereas, this relation did not occur at 2.5 μM and higher concentrations. The similar profile of PCs after lead treatment for *P. sativum* and *P. vulgaris* presented in Figs. 7 and 8 was observed by Haag-Kerwer (1999) in leaves of *B. juncea* treated with Cd^{2+} ions (25 μM $CdNO_3$). The synthesis of first PCs (mainly PC_3 and PC_4) occurred after 5 h of exposition and the level of phytochelatins increased reaching the maximum value after 72 h of treatment. The level of Cd accumulation was monitored in leaves of Indian mustard, too, and it was found that the highest change in the level of accumulated metal (almost 4 times) took place between the 24 and 48 h of exposition. During further incubation the accumulation of ions decreased slightly. This proves that the level of free Cd ions in cytosol was very high between the 48 and 72 h and maintained the PC

synthase in the state of maximum activity (this time the level of PC increased 3 times). During further exposition a decrease in PC synthase rate was observed as a result of binding Cd^{2+} ions and their accumulation in cell compartments. De Knecht et al. (1994) observed differences in the level of phytochelatins between Cd-tolerant and Cd-sensitive ecotypes of *S. vulgaris*. They noticed a lower PC content in tolerant plants (De Knecht et al., 1994). The authors explained that as having been caused by a faster transport of phytochelatin–metal complexes from the cytosol to vacuoles which limited the presence of free metal ions that can activate cytosolic phytochelatin synthase.

Other authors (Zenk, 1996; Rauser, 1995; Sanita di Toppi and Gabbrielli, 1999; Cobbett, 2000) claim that phytochelatins play an important part in maintaining the homeostasis of essential trace nutrients (such as Fe and Cu), in sulphur metabolism and also in detoxification of heavy metals.

Experiments with *cad1* mutants of *Arabidopsis thaliana* conducted by Howden et al. (1995) proved the

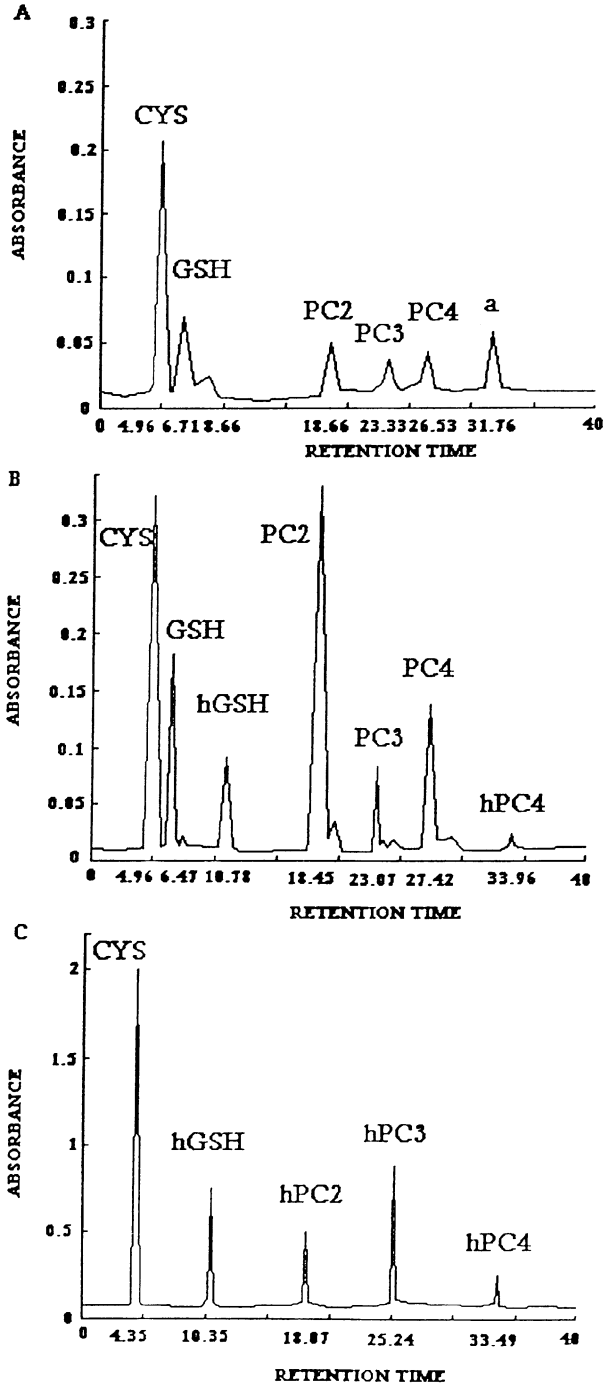

Fig. 5. HPLC chromatogram of root extract of legumes grown hydroponically in Hoagland medium with $Pb(NO_3)_2$. (A) *Vicia faba* treated with Pb for 8 h; (B) *Pisum sativum* treated with Pb for 72 h; (C) *Phaseolus vulgaris* treated with Pb for 72 h; Cys—cysteine, GSH—glutathione, h-GSH—homoglutathione, PC—phytochelatins, hPC—homophytochelatins.

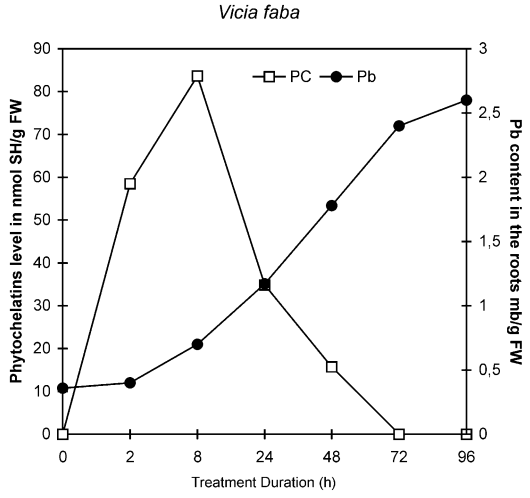

Fig. 6. The effect of lead accumulation on changes of phytochelatin contents in roots of *Vicia faba* treated with 1 mM $Pb(NO_3)_2$ for 96 h. Thiol concentration is expressed in nmol SH $\times$ g^{-1} FW.

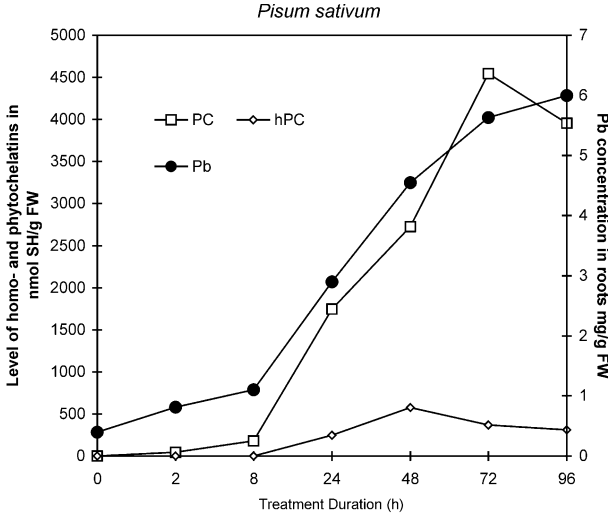

Fig. 7. The effect of lead accumulation on changes of phytochelatin and homophytochelatin contents in roots of *Pisum sativum* treated with 1 mM $Pb(NO_3)_2$ for 96 h. Thiol concentration is expressed in nmol SH $\times$ g^{-1} fr. wt.

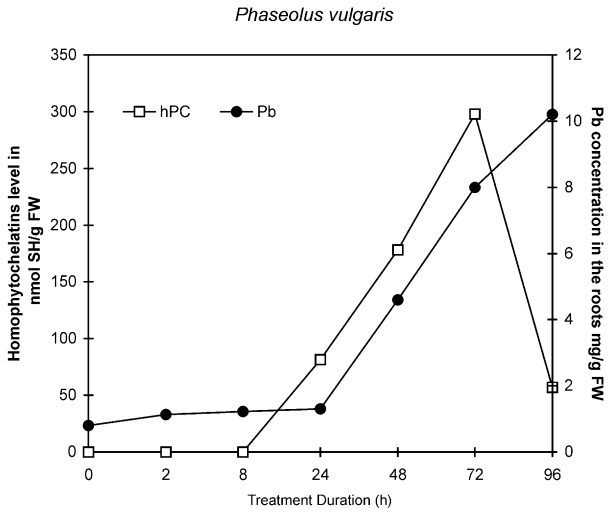

Fig. 8. The effect of lead accumulation on changes of homophytochelatin contents in roots of *Phaseolus vulgaris* treated with 1 mM $Pb(NO_3)_2$ for 96 h. Thiol concentration is expressed in nmol SH $\times$ g^{-1} fr. wt.

function of PCs in inactivation of heavy metals. It was found that these *cad1* mutants, which are unable to synthesise phytochelatins, exhibited extreme sensitivity to Cd^{2+} (already at 0.3 μM Cd^{2+} ions).

It is very likely that not only this internal "phytochelatin" detoxification system is connected with the mechanism of plant tolerance to lead, but probably there is also another mechanism, e.g. accumulation on the root surface by binding with polysaccharides (Seregin and Ivanov, 1998) or complexing with organic acids as evidenced by Harmens et al. (1994) in *S. vulgaris* which accumulated zinc. It is necessary to conduct further research in order to explain the mechanism of high plant tolerance to the effect of lead, as well as to consider the possibility of growing *P. vulgaris* varieties with an enhanced ability to accumulate Pb, e.g. as a result of adding synthetic chelates which make it possible to increase the uptake and transport of metals to the aboveground plant parts. Increasing pollution of the environment caused by heavy metals is becoming a significant problem in the modern world. Plants could be used for phytoextraction, rhizofiltration and/or phytostabilization of heavy metals. Therefore, it is necessary to recognise more broadly plant mechanisms regulating uptake and transport to aboveground plant parts, and especially tolerance to the harmful effect of heavy metals.

4. Experimental

4.1. Plant material

Seeds of three legume plants: *V. faba*, variety "Nadwislanski", *P. sativum*, variety "Sol", and *P. vulgaris*, variety "Zlota saxa", were soaked in water for 4 h and then germinated in the dark at 24 °C for 3 days. Seedlings were cultivated hydroponically in Hoagland medium supplemented with 1 mM $Pb(NO_3)_2$. The roots of 80 seedlings were exposed to 1600 ml of nutrient solution; plants were grown under controlled conditions for 96 h, at 22–23 °C for 16 h a day, with a light period providing the light intensity of 70 μmol m^{-2} s^{-1}. Roots, shoots and leaves of the tested plants were sampled after 2, 8, 24, 48, 72 and 96 h after the application of lead ions, and were rinsed for 10 min in 10 mM $CaCl_2$ and bidistilled water, and then frozen in liquid nitrogen. Samples were kept at −80 °C.

4.2. Index of tolerance

The Index of tolerance (IT) was calculated according to Wilkins (1957):

$$\mathrm{IT} = \frac{\text{average length of roots in tested solution}}{\text{average length of roots in control}} \times 100\%$$

4.3. Determining the level of lead in plant tissues

In order to determine the total amount of lead taken up by particular plant organs, 0.25 g of the plant material frozen at −80 °C was homogenised in a mortar and pestle with 5 ml 40% HNO_3. Then, the extract was warmed until a clear solution formed, 1 ml of H_2O_2 was then added and the extract was heated again. The content of lead in samples was measured with the use of the Varian Spectr. AA 20 plus atomic absorption spectrometer equipped with a deuterion lamp for background correction.

4.4. Determining the level of lead in compartments of root cells

This was done by means of homogenising 0.5 g of fresh rinsed material with 50 mM Tris–HCl pH. 7.4. The extract was centrifuged at 10 000 *g* at 4 °C for 60 min. Supernatant was collected and acidified with concentrated nitric acid. The pellet was dissolved in 5 ml 40% HNO_3 and heated again and then the content of lead in particular fractions of root cells was determined.

4.5. Determining the uptake of lead ions from Hoagland nutrient medium

Four millilitres of solution were taken from Hoagland nutrient medium containing 1 mM $Pb(NO_3)_2$, and acidified. The level of Pb^{2+} in the sample was measured with the use of the AAS method.

4.6. HPLC analysis of the content of thiol compounds

Frozen plant material was homogenised at 1:4 ratio with 5% sulfosalicylic acid. The homogenate was centrifuged twice for 5 min at 10 000 *g* at 4 °C. Separation of thiol peptides was conducted on a Nucleosil C-18 column in the linear gradient of acetonitrile in 0.1% TFA, from 0 to 40% for 40 min in Beckman high-performance liquid chromatography (HPLC). A 250 μl sample was put on the 4.6 × 250 mm Nucleosil 10 μm C-18 column. Thiol compounds were determined by the postcolumn reaction with Ellman reagent and measuring absorption at $\lambda = 412$ nm (Ellman, 1959).

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