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The Use of Pisum sativum L. As Bioindicator for Lead Ions Detoxification

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Abstract— Some plants show the ability to accumulate large amounts of lead without visible changes in their appearance or yield quality. This study aims to use of three pea (*Pisum sativum* L.) cultivars (Little Marvel, Perfection and Alderman) in lead accumulation supplemented in soil by their roots, stems and leaves. Data showed that roots of pea cultivar Perfection were highly accumulated of lead being 90 mg Pb/g fresh weight than other parts stems and leaves, where only traces of lead reached to these parts being 5.2 and 1.1 mg Pb/g fresh, respectively. The highest rate of Pb ions uptake from the soil supporting of pea root cv. Perfection took place during the first 24 h of treatment with lead being 90 mg Pb/g fresh and after 72, 96, 120 h of incubation lead content in the medium decreased by 60, 45, 40 mg Pb/g fresh, respectively. Seeds of pea cv. Perfection recorded 0.9 mg Pb/g fresh lead, while other cultivars Little Marvel and Alderman recorded 2.3 and 2.1 mg Pb/g fresh lead, respectively. This research concluded that *P. sativum* L. cv. Perfection could be used as detoxificator organisms which it can absorb pollutants from soil contaminated with lead ions.

Index Terms— Accumulation, detoxification; lead ions, pea cultivars, roots, stems, leaves, seed quality.

1 Introduction

INCREASING pollution of the environment caused by heavy metals is becoming a significant problem in the modern world. Plants could be used for phyto-extraction, rhizosphere filtration and/or phyto-stabilization of heavy metals [6]. Therefore, it is necessary to recognize more broadly plant mechanisms regulating uptake and transport to aboveground plant parts, and especially tolerance to the harmful effect of heavy metals.
Many plants can endure a level of environmental pollution that might be even several times higher than the level observed now a day. On the other hand, 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 in tissues, special plants can be used in the process of soil remediation and rhizosphere filtration. There are well-known plants that are hyper-accumulators of metals, which can accumulate metals in their leaves and stems up to a concentration of 0.1 % of dry mass [3]. The hyper-accumulator 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). Some of recognized plant species are hyper-accumulators of lead [4], in which lead concentration exceeds 0.1 % of dry wt. Kumar et al. [10] 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 l00 mg Pb/g dry wt after 20 days of cultivation in a substrate containing 625 g Pb/g dry wt.

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The level of lead accumulation in fabaceae plants can't exceed in their seeds than the maximum level permissible for man and without having any negative effect on their growth or yield [15]. Tomaszewska et al. [13] showed 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 phyto-chelatins from glutathione as the only substrate. On the other hand, lead tolerance strategies of these plants have not been fully explained yet.
In this research, trials to find a correlation between lead accumulations in root cells with their transport from roots to aboveground plant organs. I examined three Pisum sativum L. cultivars of the Fabacea family: (Little Marvel, Perfection and Alderman) and with the focus on lead accumulation in their parts (roots, stems, leaves, seeds) in order to establish if they could be used to clean soil in rhizosphere filtration processes.

2 Materials and Methods

2.1 Experimental design

Seeds of three cultivars of pea plants (*Pisum sativum* L.) cultivars (Little Marvel, Perfection and Alderman) were soaked in water for 4 hours then germinated in soil during the beginning of December, 2011 close to College of Sciences and Arts at mekhwah, Baha, KSA. Pots cultivated with pea seeds were supplemented with 1 mM $Pb(NO_3)_2$ through irrigation water 200 mg/pot. Each pot contains 5kg soil with 1m length x 1m width x 1/2m height. The 30 seeds/ pot were exposed to Pb solution. Plants were grown under controlled conditions for whole growth season with normal sunlight period. Roots, shoots and leaves of the tested plants were sampled each week 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.

2.2 Root tolerance index

The root tolerance index (TI) was calculated according to Wilkins [16]:

$$TI = \frac{\text{average length of treated roots x 100\%}}{\text{average length of roots in control}}$$

2.3 Determining the level of lead in plant organs

In order to determine the total amount of lead taken up by particular plant organs (roots, stems, leaves, seeds), 0.25 g of frozen plant material was homogenized 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 atomic absorption spectrometer (Perkin-Elmer 3030).

2.4 Statistical analysis

All data were analyzed using analysis of variance (ANOVA) procedures appropriate for a random factorial design. Mean differences among the time of testing samples were evaluated by the Least Significant Difference (LSD) method at $P < 0.05$ level of significance. All statistical analyses were performed using the software developed by the SPSS (ver. 11).

3 Results

3.1 General symptoms

This study showed no visible changes were observed in the appearance of the above-ground parts of plants after the 5th weeks of treatment with lead metal ions. In comparison to the control pea cultivars 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 color 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.

3.2 Accumulation and deotoxification by roots

Data showed that roots were the main accumulations site of Pb in the plant cultivars me studied (Table 1), which is consistent with the earlier data concerning other plants. A bulk of growth in the level of the accumulated metal was observed in the 1st week reached 81, 90 and 78 mg Pb/g fresh wt of Little Marvel, Perfection and Alderman Cultivars, respectively. Starting from the 2nd week until the end of the growth periods, steady little increases in the concentration of lead in roots for all cultivars were observed. At the same time, roots of Little Marvel and Alderman Cultivars were accumulated lead lesser than Perfection. In the 2nd week of cultivation pea cultivars with Pb $(NO_3)_2$, I found about 8, 10 and 16 mg Pb/g fresh wt in roots of Little Marvel, Perfection and Alderman Cultivars, respectively. The TI values of the examined pea cultivars plants were significantly different (Table 2) after 1st week of lead treatment. Although Perfection cultivar exhibits the highest sensitivity to

Table 1: The lead content (mg Pb/g fresh weight) of pea roots cultivars grown in soil supplemented with 1-mM Pb $(NO)_3$.

Weeks	Pb content of pea cultivars (mg Pb/g fresh weight)		
	Lit. Marv.	Perf.	Alder.
1st week	81	90	78
2nd week	85	95	86
3rd week	95	100	92
4th week	99	102	95
5th week	101	109	99
LSD (P<0.05)	3.3	4.2	2.4

Table 2: Tolerance index (%) estimated by means of the Wilkins' test for the lead content of pea roots cultivars grown in soil supplemented with 1-mM Pb $(NO)_3$.

Weeks	Root tolerance index of pea cultivars (%)		
	Lit. Marv.	Perf.	Alder.
1st week	35.33	33.10	31.00
2nd week	45.00	36.10	41.23
3rd week	54.00	45.40	48.21
4th week	67.00	53.50·	58.31
5th week	78.70	62.00	61.00
LSD (P<0.05)	12.3	12.4	12.1

lead ions (TI = 22%), it has the capacity to take up the largest amount of Pb. In the 5th week, similar TI for both Perfection and Alderman Cultivars being 62 and 61%, respectively, while Little Marvel recorded 78.70%.

3.3 Levels of Pb ions in stems and leaves

The level of lead in stems and leaves of all the studied pea cultivars plants increased with the time of lead treatment (Table 3 and 4). In stems the maximum level of Pb in Little Marvel cultivar was 5.1 mg Pb/g fresh wt, followed by Alderman cultivar was 5.0 mg Pb/g fresh wt, and in Perfection cultivar was 1.1 mg Pb/g fresh wt (Table 3). These values were lower than the lead content in the roots by many times.

Table 3: The lead content (mg Pb/g fresh weight) of pea stems cultivars grown in soil supplemented with 1-mM Pb $(NO)_3$.

Weeks	Pb content of pea cultivars (mg Pb/g fresh weight)		
	Lit. Marv.	Perf.	Alder.
1st week	5.1	1.1	5.0
2nd week	5.6	1.1	5.2
3rd week	5.8	1.2	5.3
4th week	5.9	1.5	5.8
5th week	7.8	2.2	6.6
LSD (P<0.05)	1.1	0.2	1.2

A significant increase in lead content was observed in leaves of Little Marvel, Perfection and Alderman Cultivars after the 3rd week (Table 4). The highest amount of lead was found in leaves of Little Marvel cultivar being 1.32 mg Pb/g fresh wt. Lower contents of accumulated Pb were found in leaves of Perfection pea cultivar. The maximum levels in leaves of Perfection pea cultivar did not exceed 0.4 mg Pb/ g fresh wt.

Table 4: The lead content (mg Pb/g fresh weight) of pea leaves cultivars grown in soil supplemented with 1-mM Pb $(NO)_3$.

Weeks	Pb content of pea cultivars (mg Pb/g fresh weight)		
	Lit. Marv.	Perf.	Alder.
1st week	1.23	0.01	1.02
2nd week	1.24	0.01	1.03
3rd week	1.31	0.03	1.06
4th week	1.32	0.03	1.06
5th week	1.32	0.04	1.06
LSD (P<0.05)	0.3	0.2	0.4

3.4 Seed quality

The final step in this research was recording changes in seeds characters of studied cultivars in response to lead ions treated in the soil medium (Table 5). Roots of Perfection pea cultivar showed the best capacity to absorb and taking up lead ions from the nutrient soil medium to seed reached to minimum being 0.1 mg Pb/g fresh wt. Similarly as in the case of Little Marvel and Alderman cultivars the rate of uptake was falling during a longer exposition time and in the time interval 4-5 weeks of cultivation pea roots took up only further 23% of Pb in the medium. Yield of the investigated Perfection pea plant cultivar (301.4g) increased to be closed to the values of control (313.4g). Simultaneously, seed number/plant recorded 68 of Perfection pea under Pb treatment while control recorded 71. Seed number/pod and is not varied. The percentage of seed weight were similar for both Little Marvel and Alderman cultivars 139.9 and 138.4, while Perfection pea plant cultivar recorded the highest values being 163.1.

Table 5: The seed lead content (mg Pb/g fresh weight) and yield components of pea cultivars grown in soil supplemented with 1-mM Pb $(NO)_3$.

Seed characters	0-mM Pb in soil			1-mM Pb in soil		
	Lit.	Per	Ald	Lit	Per	Ald
Seed Pb (mg/g)	0.0	0.0	0.0	1.3	0.1	1.1
Yield/pot (g)	31	31	311	28	30	299
	2	3		9	1	
Seed number/pod	5	6	6	5	5	5
Seed number/plant	65	71	68	61	68	62
Seed % (g)	14	17	143	14	16	138
	5	0		0	3	

4 Discussion

A few unfavorable changes in the appearance of the Pb-treated roots in examined cultivars of pea plants under Pb application in soil. For example browning, the lowered number of hair roots, growth inhibition and a decreased biomass growth were recorded. An unfavorable effect of heavy metal ions, for example Pb, on roots was also observed by other researchers. For example, Wierzbicka [15] 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 with heavy metals. Geebelen et al. [9] found a visible reduction of the root growth in P. vulgaris. This was correlated with a decrease in the root mass by as much as 90%. Seregin and Ivanov [12] 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 [8] observed changes in the appearance of roots correlated with a decrease in dry and fresh mass after lead treatment. Due to roots could provide the primary route for the penetration of heavy metal ions. Roots can take up 90-95 times more lead than stems and leaves [17]. Kumar et al. [10] demonstrated that about 90% of Pb accumulation occurred in roots. The amount of lead ions in the roots of the studied Perfection is comparable with the results obtained by Burzynski [5], who exposed P. vulgaris, cucumber and wheat to 10-3 M Pb-ion. 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 only 24 h of exposition. He also demonstrated that *Phaseolus vulgaris* accumulated over 75 mg Pb/g dry wt in roots and exhibited a significant tolerance to this metal [5]. Antosiewicz and Wierzbicka [2] localized 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 [12] 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 [12]. Leopold and Gunther [11] observed in Silene vulgaris cell cultures that only 5% of the total amount of metal ions was present in a soluble extract. It is thought to be some these plants possess a mechanism which limits the translocation of metals to shoots [1]. There are some unique plants-metallophytes which are hyperaccumulators that accumulate a high amount of metals in shoots [1]; [3]. The unique ability to accumulate lead, which is especially characteristic of pea cultivar especially Perfection, could be exploited in rhizosphere filtration of lead from polluted soil. One cannot exclude the fact that genetic manipulations might lead to creating a variety 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 EOTA, both into soil [7] and hydroponic cultures [14] raised the concentration of soluble lead and increased its uptake by the plant by creating Pb-complexes. These results suggest that further studies should be conducted on the effect of this chelator on lead uptake by Perfection cultivar.

5. Summary

The presented results showed that about 90-95% of the total amount of lead was localized in roots and only 5-10% was transported to the above-ground plant parts in all three pea plants cultivars after 5 days of lead treatment. Differences between TI values indicate that Little Marvel has the highest resistance among the three plant cultivars and has the fastest initiation of the detoxicative system. Roots of Perfection lowered the lead concentration in the soil medium by half of the initial value within the first hours, whereas, during the next days of exposition, the rate of metal uptake was significantly slower.

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