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Removal of Lead Ions from Polluted Water Using *Plantago major* L. and *Phaseolus vulgaris* L.

Akram A. Ali and Ali A. AL-Homaidan

Department of Botany and Microbiology, Faculty of Science,
King Saud University, P.O. Box: 2455, Riyadh 11451, Kingdom of Saudi Arabia.

Abstract: This study focuses on lead detoxification using roots, stems, leaves and whole plants of *Plantago major* L. and *Phaseolus vulgaris* L. (decoration kidney bean) as a ball hanging hydroponically in deionized water supplemented with concentrations of lead ion under different duration times and temperatures regimes. Roots of studied plants showed that the highest removal of lead than other parts, while using a mixture of whole plants were more efficient than using each one alone. The largest amounts of lead 85, 77 and 71 % were accumulated in roots of *P. major* + *P. vulgaris*, *P. major* alone and *P. vulgaris* alone, respectively. The highest rate of Pb ions uptake from the medium took place during the first 2-days of incubation and after 5-days of incubation, lead content in the medium decreased by half. This study suggested that roots of *P. major* + *P. vulgaris* could be used removal of pollutants from water contaminated with lead.

Keywords: Detoxification; lead ions; hyperaccumulative plants, biosorption

INTRODUCTION

Human activities lead to Increase concentrations of heavy metals like lead in soil, water, air and living organisms caused by human activities (Seregin and Ivanov 2001). Elevated levels of heavy metals in water are taken up by the root system, accumulate in different parts of plants, reduce their growth and impair plant metabolism (Allinson and Dzialo 1981). On the other hand, introduction of these metals to man and animals through drinking water will help in occuring many diseases to them. Lead is one of the most widely distributed heavy metals and is very toxic to plants. Its effect on plants has been studied by several research terms (Bazzaz *et al.,* 1974; Titov *et al.,* 1996), but most work has been done on isolated systems.

The hyperaccumulator plants include about 400 species (Morel *et al.,* 1997). They are well-known and usually show the ability to accumulate 1000 times more heavy metal (lead) than normal plants without visible changes in their appearance or yield (Baker, 1995). At the same time, the level of lead accumulation can exceed even several hundred times the level permissible for living organism. Thus, lead will be introduced into the food chain and biological circulation. Therefore, the use of non-edible plants is preferable (Kucharski *et al.,* 1999).

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 (Maitani *et al.,* 1996). Very often *P. major* and *P. vulgaris* plants grow in soil containing a high Pb concentration. From the results of various studies, Sheoran and Singh (1993) and Grill *et al.* (1989) have proposed several sites in the electron transport chain that are influenced by heavy metal. In the whole plant, Pb can affect photosynthesis at the stomatal level, mesophyll cells, pigment content, and light and dark reactions. Maitani *et al.* (1996) have shown that it affects chlorophyll biosynthesis in barley seedlings. At a high Pb content in soil, photosynthesis can also be reduced due to both a lower carboxylase activity and the effects on metabolites of the carbon reduction cycle (Stiborova *et al.,* 1986, 1987). As a result of reduced photosynthesis, the effect of Pb is perceived as a decrease in plant growth and development. A direct effect of Pb on stomata was described by Carlson *et al.* (1995).

Studies on the use of air dried balls from *P. major* and *P. vulgaris* are new. These plants widely distributed in

Corresponding Author: Akram A. Ali, Department of Botany, Faculty of Science, Zagazig University, Zagazig, Egypt.
E-mail: akram692000@yahoo.com

natural and cultivated habitats. Different strategies for lead in environmental stress conditions by one or more than one organs of them may occur. To clarify which parts of the plant control the stability to increased Pb concentrations in water, we have studied its influence on the concentration of lead in water. This study is divided into three stages: first stage to select the best concentration from lead ion treatments; second is to determine the best duration to plant samples to take up the biggest amount; the last to show the effect of different temperatures on Pb-ion absorption.

MATERIALS AND METHODS

Experimental and Treatments:

Seeds of *Plantago major* L. and *Phaseolus vulgaris* L. (decoration kidney bean) were planted in pots under controlled conditions from suitable soil and irrigation. These plants were left until reaching good vegetative growth (before flowering). We divided plants into four groups: roots, stems, leaves and whole plants. These groups were left in air till complete dryness. Air dried groups of the tested plants were arranged in balls as: roots of *P. major* (100 gm); stems of *P. major* (100 gm); leaves of *P. major* (100 gm); whole plant of *P. major* (100 gm); roots of *P. vulgaris* (100 gm); stems of *P. vulgaris* (100 gm); leaves of *P. vulgaris* (100 gm); whole plant of *P. vulgaris* (100 gm); mixture roots of *P. major* + *P. vulgaris* (50 gm + 50 gm); mixture stems of *P. major* + *P. vulgaris* (50 gm + 50 gm); leaves of *P. major* + *P. vulgaris* (50 gm + 50 gm)*;* mixture whole plant of *P. major* + *P. vulgaris* (50 gm + 50 gm). These balls were hanged (using iron rode) hydroponically in water supplemented with 1, 2, 3, 4 and 5 mM $Pb(NO_3)_2$. Samples were tested after 1, 2, 3, 4 and 5 days for their lead ion content under different temperature regimes (10, 20, 30, 40, and 50 ^oC).

Analysis:

In order to determine the total amount of lead taken up by particular plant organs and whole plants, 0.5-g of the plant material was homogenized in a mortar and pestle with 5- mL of concentrated HNO_3 and concentrated H_2SO_4 (1:1 volume by volume). 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 Varian Spectrophotometer AA 20 plus atomic absorption spectrometer equipped with a deuterion lamp for background correction.

Statistical Analysis:

Statistical analyses were carried out using the SPSS BASE 10.0 (SPSS Inc., Chicago, IL) packages, to testify the significant effects of air dry balls of *P. major* and *P. vulgaris* to different concentrations on lead ions. Plant parts uptake ions were subjected to normal procedures of analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

Results:

In this research, materials were two plants: *P. major* L. and *P. vulgaris* L. (decoration kidney bean). These plants were grown hydroponicaily in a water medium supplemented with lead ions under different temperature for certain times. The roots of studied plants showed high uptake to large amounts of sublethal 10^{-3} M Pb in water medium (Table 1). Fewer uptakes were observed in the above-ground parts of plants after the third day of treatment with lead ions (Table 2). In comparison to the control *P. major* roots grown under 10^oC, the roots of the *P. vulgaris* plants and the mixture roots of both plants changed significantly (Table 3). We observed gradual changes in the Pb-uptake reaching maximum especially, in the 40^oC of organs in the plants treated with lead. The absorption of the roots changed gradually and stabilized after the forth day under the influence of metal ions, from 65% to 79%. The uptake of roots was slowed down in samples treated with other than the 1 mM Pb-ion concentrations and the number of hair roots decreased, which led to lowered water uptake (Table 1). Responses differences between plant organs and whole plants to different Pb-ion values indicate that the lowest concentration of lead ion is the best among the rest of concentrations and has the fastest initiation of the detoxicative system. The *P. major* exhibits the highest sensitivity to lead ions and it has the capacity to take up the largest amount of Pb (66%). We found that roots have the highest accumulation capacity of Pb in the tested plants (Table 1),

Table 1: Removal of lead content (%) by balls of *Plantago major* L. and *Phaseolus vulgaris* L. hanged and left it in water (with 25°C) for five days.

Lead concentrations Plant parts	1-mM $Pb(NO_3)_2$	2-mM $Pb(NO_3)_2$	3-mM $Pb(NO_3)_2$	4-mM $Pb(NO_3)_2$	5-mM $Pb(NO_3)_2$
Effect of roots ball:					
Plantago major	77	63	66	69	44
Phaseolus vulgaris	71	67	61	69	41
Plantago + Phaseolus	85	76	65	72	55
LSD ($P \leq 0.05$)	5.1	7.8	6.8	4.1	8.6
Effect of stems ball:					
Plantago major	56	52	51	52	50
Phaseolus vulgaris	65	54	54	54	45
Plantago + Phaseolus	55	45	43	41	42
LSD ($P \leq 0.05$)	6.6	9.7	7.9	9.7	6.6
Effect of leaves ball:					
Plantago major	46	44	42	44	46
Phaseolus vulgaris	52	40	42	40	42
Plantago + Phaseolus	50	43	40	43	40
LSD ($P \leq 0.05$)	5.9	7.0	9.9	7.0	5.9
Effect of whole plant ball:					
Plantago major	66	44	46	44	56
Phaseolus vulgaris	58	50	50	50	48
Plantago + Phaseolus	57	50	47	50	47
LSD ($P \leq 0.05$)	9.0	11.2	9.8	11.2	9.0
Effect of plant organs ball:					
Roots *Plantago major*	77	63	66	69	44
Roots *Phaseolus vulgaris*	71	67	61	69	41
Stems *Plantago major*	56	52	51	52	50
Stems *Phaseolus vulgaris*	65	54	54	54	45
Leaves *Plantago major*	46	44	42	44	46
Leaves *Phaseolus vulgaris*	52	40	42	40	42
LSD ($P \leq 0.05$)	8.8	9.7	7.5	9.7	8.8

Table 2: Removal of lead content (%) by balls of *Plantago major* L. and *Phaseolus vulgaris* L. hanged in water (with 25°C) treated with 1 mM of $Pb(NO_3)_2$.

Treatments duration Plant parts	After 1-day	After 2-days	After 3-days	After 4-days	After 5-days
Effect of roots ball:					
Plantago major	68	75	76	77	77
Phaseolus vulgaris	65	70	71	71	72
Plantago + Phaseolus	74	75	79	79	79
LSD ($P \leq 0.05$)	6.8	9.8	9.6	6.1	6.1
Effect of stems ball:					
Plantago major	46	52	55	55	56
Phaseolus vulgaris	45	54	54	54	54
Plantago + Phaseolus	44	55	55	55	58
LSD ($P \leq 0.05$)	6.9	6.7	6.9	6.9	6.8
Effect of leaves ball:					
Plantago major	42	44	55	55	55
Phaseolus vulgaris	41	42	50	52	55
Plantago + Phaseolus	40	43	49	50	50
LSD ($P \leq 0.05$)	6.3	7.7	6.9	3.5	3.7
Effect of whole plant ball:					
Plantago major	55	63	66	66	66
Phaseolus vulgaris	51	57	60	67	67
Plantago + Phaseolus	50	60	67	70	70
LSD ($P \leq 0.05$)	9.7	5.4	7.3	6.3	6.3
Effect of plant organs ball:					
Roots *Plantago major*	68	75	76	77	77
Roots *Phaseolus vulgaris*	65	70	71	71	72
Stems *Plantago major*	46	52	55	55	56
Stems *Phaseolus vulgaris*	45	54	54	54	54
Leaves *Plantago major*	42	44	55	55	55
Leaves *Phaseolus vulgaris*	41	42	50	52	55
LSD ($P \leq 0.05$)	4.6	6.4	3.5	5.3	7.2

Table 3: The Removal of lead content (%) by balls of *Plantago major* L. and *Phaseolus vulgaris* L. hanged in water for five days and treated with 1mM of $Pb(NO_3)_2$.

Water temperaturesPlant parts	10°C	20°C	30°C	40°C	50°C
Effect of roots ball:					
Plantago major	34	62	77	87	53
Phaseolus vulgaris	44	66	71	77	44
Plantago + Phaseolus	49	66	82	91	45
LSD ($P \leq 0.05$)	6.4	4.6	5.3	6.2	4.2
Effect of stems ball:					
Plantago major	32	41	54	65	41
Phaseolus vulgaris	31	44	54	64	39
Plantago + Phaseolus	29	43	49	55	42
LSD ($P \leq 0.05$)	7.3	6.8	5.6	8.6	9.3
Effect of leaves ball:					
Plantago major	33	39	44	54	38
Phaseolus vulgaris	30	41	46	51	44
Plantago + Phaseolus	36	53	66	68	55
LSD ($P \leq 0.05$)	7.1	4.5	6.3	3.6	6.8
Effect of whole plant ball:					
Plantago major	44	49	56	66	55
Phaseolus vulgaris	42	45	56	65	43
Plantago + Phaseolus	51	56	59	62	55
LSD ($P \leq 0.05$)	4.4	5.5	6.4	6.4	4.7
Effect of plant organs ball:					
Roots *Plantago major*	34	62	77	87	53
Roots *Phaseolus vulgaris*	44	66	71	77	44
Stems *Plantago major*	32	41	54	65	41
Stems *Phaseolus vulgaris*	31	44	54	64	39
Leaves *Plantago major*	33	39	44	54	38
Leaves *Phaseolus vulgaris*	30	41	46	51	44
LSD ($P \leq 0.05$)	5.2	5.0	6.3	4.3	5.4

which is consistent with the earlier data concerning other plants. A steady increase in the level of the biosorped metal was observed in all samples after the first day of treatments until the end of the experimental period when the concentration of lead in roots of *P. major* + *P. vulgaris* reached 79 % (Table 2). At the same time, roots of *P. major* and *P. vulgaris* uptake lead to a lesser degree (77%, 72%, respectively). After 40°C of water temperature treated with $Pb(NO_3)_2$,we found about 50% of Pb/ in roots were missing (Table 3).

The presented results showed that about 90-95% of the total amount of lead was localized in roots after the first day of treatments and only 5-10% was transported to it in all after 4-days of lead treatment (Table 2). The level of lead in stems and leaves of all studied plants increased with the time of lead treatment but they were stable in the fourth day (Table 2). In stems, the maximum level of Pb in studied organs is varied. These values were, 58% (*P. major* + *P. vulgaris*), 56% (*P. major*) and 54% (*P. vulgaris*) and lower than the lead content in the roots by 25%. A significant increase in lead content was observed in leaves of *P. major* and *P. vulgaris* after 3-days compared to the 1-days or 2-days, while it observed in leaves of *P. major* + *P. vulgaris* after 4-days compared to the 1-days or 2-days (Table 2). The highest amount of lead was found in leaves of *P. major* + *P. vulgaris* 50% (Table 1). Lower contents of biosorped Pb were found in leaves of bean.

Discussion:

Increasing pollution of the environment caused by heavy metals is becoming a serious problem in the modern world. Plants could be used for phytoextraction, rhizofiltration and/or phytostabilization of heavy metals. Therefore, it is necessary to recognize more broadly plant mechanisms regulating uptake using below or aboveground plant parts, and may lead to reducing the harmful effect of heavy metals.

It is a well-known that roots provide the primary route for the penetration of heavy metals. Roots can take up 3-50 times more lead than stems or leaves (Wozny *et al.,* 1995). The mixture plants we had studied uptake up to 85% Pb in roots and this is similar to results of Tomaszewska *et al.* (1996). 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 only one day 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) 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 (2001) 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, 2001). 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.

The investigated plants: *P. major* and *P. vulgaris* showed much variation in absorbing lead. The *P. major* biosorped much higher amounts of Pb than *P. vulgaris*. In comparison, the content of this metal in roots of Brassicacea, which is thought to be a moderate uptake 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 77% of *P. major,* and 71 % of *P. vulgaris.* The best accumulator among the studied samples of the research was the mixture roots of both tested plants recording 85 %.

Biosorption of lead is characteristic to *P. major* than *P. vulgaris,* could have higher phytoextraction ability of lead from polluted water. One can't exclude the fact that genetic manipulations might lead to creating a variety of *P. major*. Moreover, in the other research on lead accumulation in *Brassica juncea,* it has been demonstrated that application of synthetic chelators (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- complexes. These results suggest that further studies should be conducted on the effect of this chelator on lead uptake by *P. major.* Similarly, a significant increase in lead accumulation occurred in the roots of *Brassica 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.

The gradual uptake of lead in studied organs: roots, stems, and leaves of *P. major* than *P. vulgaris* and reached maximum after the second day and 40°C may suggest that phytochelations 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 metal complexes disappeared between the 7th and 14th day of treatment. According to these authors, this proves that the formation of phytochelation-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 to an increased tolerance of plants to metals. Leopold and Gunther (1997) and Leopold *et al.* (1999) also analyzed extracts of cell cultures and plants and they observed that eluants from HPLC column were complexes of Cd and Cu ions with legends (molecular weight about 13000 Da), which correspond to the phytochelation molecule 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 legends (they could not be sufficiently purified for mass spectrometry), but not phytochelations. 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, 2001) or complexing with organic acids as evidenced by Harmens *et al.* (1994) in *S. vulgaris* which accumulated zinc.

This study ensure that 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. major* varieties with an enhanced ability to uptake Pb, e.g. as a result of adding different accumulative plants which make it possible to increase the uptake of metals with help of different plant parts.

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