

EFFECT OF Pb ON GERMINATION OF DIFFERENT SEED AND HIS TRANSLOCATION IN BEAN SEED TISSUES DURING SPROUTING

S. Zoran Ilić^{1,*}, Nataša Mirecki², Radmila Trajković³, Nikolaos Kapoulas⁴, Lidija Milenković¹ and Ljubomir Šunić¹

¹ Faculty of Agriculture Priština-Lešak, Kopaonička bb, 38219 Lešak, Serbia

² Biotechnical Faculty, University of Montenegro, Podgorica, Montenegro

³ Faculty of Natural Sciences and Mathematics, Priština, 38220 Kosovska Mitrovica, Serbia

⁴ Regional Development Agency of Rodopi, Plastera K. Antoniad, 69100 Komotini, Greece

ABSTRACT

The effect of treatment of seed with various lead concentrations of Pb-acetate (10^{-5}M , 10^{-4}M , 10^{-3}M , 10^{-2}M and $2 \times 10^{-2}\text{M}$) on seed germination (beans, onion, raddish, carrot, lettuce, brussels sprout) was investigated. The distribution of lead (Pb) in different tissues of the bean seed (seed coat, endosperm, embryo), depending on seed mass (large, medium and small) was also determined. We found that the highest concentration of Pb-acetate ($2 \times 10^{-2}\text{M}$) causes considerably higher loss of germination in almost all the vegetable species studied, while in carrot and lettuce it was totally eliminated. The highest tolerance in regard to the increased concentrations of Pb-acetate on seed germination was found in the seeds of bean and onion. Lower applied concentrations of Pb (10^{-5}M , 10^{-3}M) did not significantly accumulate and mobilize lead in endosperm (0.22 and $5.50 \mu\text{g g}^{-1}$) and embryo (5.33 and $20.52 \mu\text{g g}^{-1}$ in medium and large size of seed). The seed coat accumulated significant amounts of Pb at the highest Pb-acetate concentration ($2 \times 10^{-2}\text{M}$). The Pb content in seed coat was highest ($1139 \mu\text{g g}^{-1}$) in seeds with small total mass, with the lowest accumulation of Pb ($1052 \mu\text{g g}^{-1}$) observed in the largest seed. Accumulation of Pb in the seed coat is a form of protection mechanism from the unfavorable effects of high concentrations and lead accumulation in the central parts of the seed (embryo). Seeds with the largest total mass accumulate less content of Pb in embryo and endosperm respectively.

KEYWORDS:

Pb, seed, germination, bean, distribution

1. INTRODUCTION

Plants absorb heavy metals from the soil through the root and from the atmosphere through over ground vegetative organs [1]. The quantity or level of heavy metals ab-

sorption in plants, depends not only on the concentration of the metals in the physical and chemical composition of the soil [2], but also varies in different plant species [3]. Some authors have reported the existence of differences in accumulation of heavy metals in plant cultivars, organs, and tissues [4]. Lead is not essential for higher plants, and if plants need Pb, the needed concentration ranges between 2 and 6 ppb [5]. There is, however, data that indicates that lower concentrations of Pb can stimulate plant growth [6]. It has been established that treating seeds with high heavy metals concentration reduces the biological value of the seed, as well as the growth of the primary root [7]. A number of experiments have established the unfavorable effects of high Pb concentrations on life processes in plants. For example, it was determined that Pb inhibits seedling growth [8], cell elongation [9] and photosynthesis [10], causes interruption of mitosis [11], affects the water regime [12], mineral nutrition of plants [13] enzyme activities [14, 15] and induced deficiency of CO_2 due to stomatal closure [16].

The effects of metals on seed germination from different plants depend on interspecies differences in seed structure, particularly seed coats, as seed coats have a wide range of anatomic forms that exist in no other plant organ or tissue [17]. Although the seeds are tolerant to the presence of all metals, the percentage of germinated seeds depend on the type of metal and its concentration. Numerous studies have been carried out on seed germination and seedling growth of different plant species exposed to heavy metals [18-20]. The decrease in seed germination is attributed by the heavy metal treatment [21, 22]. Lead has an inhibitory effect on seed germination. Lower concentrations of heavy metals (between 3 and 33 ppm) have been shown to partially inhibit seed germination of *Picea abies* L. and the highest concentrations (100ppm) of all metals to cause total inhibition [23]. *Vigna mungo* and *Vigna radiata* were the most tolerant on the seed germination ones than *Lens culinaris* and *Pisum sativum* to most of the heavy metals [24]. Reduced seed germination is observed in corn treated with 20, 50, 100 and 200 $\mu\text{g/ml}$ lead acetate [25]. There is a continuous decrease in the extent of germination

* Corresponding author

with the increase of lead concentration. At a 1.0 mM concentration, the germination was decreased by 10% while it reached up to 100% at 500 mM [26]. An acidic environment is an important requirement for faster penetration of lead in the seed that inhibits germination. In order to reduce the negative effect of lead, the use of heavy metal chelators such as cysteine and salts of Na and Cl tetra acetic acid EDTA, and fat soluble vitamins A, E and D, have been resorted to [27].

Lower concentrations of Pb have not significantly affected mobilization of mineral matter during seed germination [28]. Thus, the presence of low concentrations of both Pb and Cd result in a small stimulation of the growth of seedlings of buckwheat cultivars, while higher doses inhibit root growth [29]. Though the effects are more pronounced at higher concentration and duration, in some cases lower concentrations may stimulate seed germination [30].

Seed coats were impermeable to lead in the first period of imbibition when water uptake is intense. In the final stages of imbibition, when water uptake is reduced, seed coats became more permeable to lead. The lead that penetrated into the embryos in the final stage of imbibition delayed germination. This shows that seed coats are selectively permeable to lead ions [11]. Seed of *Phaseolus vulgaris* has a great absolute mass, its a large seed, easy to separate the individual parts during the germination and may be easier to follow the distribution of lead in the different seed tissue unlike of the seed with low absolute mass (onion, radish, carrot, lettuce, brussels sprout).

Having all this in mind, the aims of this paper were to determine the effects of different concentrations of Pb-acetate on germination of different vegetable seed. Large, medium and small seeds of bean should be compared in terms of metal accumulation and distribution in different seed tissues (seed coat, endosperm, embryo) during sprouting.

2. MATERIAL AND METHODS

2.1 Experiment 1: Effect of Pb on seed germination

Seeds of beans (*Phaseolus vulgaris* L.), onion (*Allium cepa* L.), radish (*Raphanus sativus* L. var. *radicola*), carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.) and brussels sprout (*Brassica oleracea* var. *gemmifera*) were obtained from the Institute of Field and Vegetable Crops from Novi Sad. All seeds were surface sterilized in 0.5% sodium hypochlorite solution for 20 min and washed thoroughly with distilled water. The seeds were germinated in Petri dishes (150 mm diameter) with double layer of filter paper soaked (10 ml) in varying concentrations of Pb-acetate (10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M and 2×10^{-2} M).

In order to reduce the negative influence of Pb the use of heavy metal chelators such as ethylenediaminetetraacetic acid (EDTA) was applied. In a second series of experiments the same seeds were treated with 10 ml of Pb-acetate at different concentrations (10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M and 2×10^{-2} M) plus 5 ml of EDTA (120 ml/ L H₂O). Distilled

water was used as control. The seeds were set under a photoperiod of 12 hr, and 25 ± 1 °C temperature. The root and shoot length of the seedlings were measured and the germination percentage was recorded after 96 hr. A 1-mm radical emergence from the seeds was considered as seed germination. Data are the results from five replications with 100 seeds in each Petri dish.

Experiment 2: Pb translocation in the seed

The experiment was carried out using the seed of bean (domestic old cultivar 'Tetovac'). Enrichment of seeds with Pb was completed by their imbibition in solutions with various concentrations of Pb-acetate (10^{-5} M, 10^{-3} M and 2×10^{-2} M) at 22 °C during 24 hours. Depending on the seed weight the samples were derived into three groups : large (715 g), medium (465 g) and small (280 g). Each sample contained the same number of seeds. Following the water uptake dynamics during 24 hours showed that at 22 °C the water content in the seed reached 40%, which is sufficient for germination and enables maximal introduction of Pb into the seed tissues. After imbibition, seeds were rinsed with distilled water. Seeds were then kept in a thermostat for germination for 4 days at 25 °C. Afterwards, the biological value of the seed was examined, according to the procedure published in Official Gazette of SFRY (Službeni list SFRJ 47/87).

To study the heavy metals accumulation in different seed bean tissue were oven dried (110 °C) for 24h. The samples (0.3 g) were digested in a 1:3 HNO₃ and HCl mixture. The digested samples were filtered through a Whatman filter (N^o.42) before metal analysis, and the concentration of lead was determined on an atomic absorber (Unicam 929).

Statistical analysis. We performed two independent experiments. Data are reported as means $\pm$ SD. Statistical significance was evaluated with Student's t-test, and considered to be significant when the P value was less than 0.05.

3. RESULTS AND DISCUSSION

Lead is not essential for higher plants. There is, however, data that indicates that lower concentrations of Pb can show stimulating effect on seed germination [27]. With the increase of Pb concentration, a tendency of decrease of germination ability, germination energy and typical seedlings are observed, along with an increase in number of atypical seedlings and non-germinated seeds. However, the obtained differences in the mentioned parameters were not statistically significant [27]. This could be because Pb mostly remained in the peripheral parts of the seed and did not reach the germ. High concentration of Pb-acetate (10^{-2} M) in lettuce seed totally prevents germination, while in seeds of the other vegetable species germinations decreased by 26% in carrots, 17.1% in brussels sprouts, and by a moderate 7.3% in radishes in relation to the control (Table 1).

TABLE 1 -The influence of Pb-acetate on the seed germination under experimental intoxications

	beans	onion	raddish	carrot	lettuce	brussels-sprout
Control (H ₂ O)	100.0	100.0	100.0	100.0	100.0	100.0
10 ⁻⁵ M Pb-acetate	101.0±0.5 ^a	98.9±1.2 ^a	102.0±0.5 ^a	98.9±0.8 ^a	100.0±0.3 ^a	103.0±0.5 ^a
10 ⁻⁴ M Pb-acetate	98.4±1.2 ^a	98.9±0.8 ^a	101.0±1.0 ^a	97.8±1.1 ^a	93.4±1.5 ^a	102.0±0.5 ^a
10 ⁻³ M Pb-acetate	97.9±1.5 ^a	96.4±1.0 ^a	101.0±1.0 ^a	93.4±1.8 ^a	88.2±2.2 ^{ab}	97.6±1.2 ^a
10 ⁻² M Pb-acetate	92.2±2.0 ^a	89.9±2.8 ^{ab}	92.7±1.6 ^a	74.0±4.1 ^b	0.00±0 ^d	82.9±2.5 ^{ba}
2 x 10 ⁻² M Pb-acetate	78.3±3.6 ^b	73.0±3.2 ^b	54.2±4.5 ^c	0.00±0 ^d	0.00±0 ^d	19.4±6.8 ^d

Our results let us conclude that beans in early phases of growth are characterized by high tolerance to toxic concentrations of Pb. Higher concentrations of heavy metals, among other Pb, can induce protein synthesis, phytochelatins, which was found in maize seedlings [30].

Doubling the concentration of Pb-acetate (2x10⁻²M) causes considerably higher loss of germination almost in all vegetable species investigated, while in some species such as carrot and lettuce germination is totally eliminated. The highest tolerance in regard to the increased concentrations of Pb-acetate is found in the seeds of bean and onion.

However, low concentrations of lead (10⁻⁴ M and 10⁻⁵ M) cause to root growth of *Brassica juncea* while root growth of *Zea mays* and *Allium cepa* were inhibited [31]. Seed germination and root development in plant was gradually reduced with the increase of Pb concentration [32; 33]. Seed germination wasn't effected from 125 µg mL⁻¹ concentrations but 250 µg mL⁻¹, 500 µg mL⁻¹ and 1000 µg mL⁻¹ concentrations inhibited to seed germination in ratio 34.33%, 43.33% and 56.67% respectively [34].

The same trend has been observed with the highest concentration of lead which causes inhibition of germination of *Pisum sativum* [35]. *Lupinus luteus* seeds lost some degree of germinability when they were exposed to lead [36]. The same trend was observed in *Orzya sativa* [37] and in *Sinapis alba* [38]. In contrast, lead did not have a significant effect on germination in *Rumex thrysiphlorus* [11]. These results support the observations reported by other authors on the effect of different metals on seed germination [39]. However, the authors implied that, the amount of lead per seed mass unit was important; suggesting that the concentration of lead is not important on germination rate. This issue may relate to permeability of seed coat to metal ions. Due to the different degree of permeability of seed coats to metals, such as lead, may result in a different degree of germination inhibition.

Acetic medium is a considerable precondition for a sooner penetration of Pb into the seed which inhibits germination. In order to reduce the negative influence of Pb the use of different chelators of heavy metals such as Na and Cl salts acetic acid (EDTA) is applied. EDTA is a chelator which reduces the toxicity of Pb by binding to it, and thus disables the inhibitory influence of Pb on the enzymes responsible for the germination.

In medium with high concentration of Pb-acetate (2 x 10⁻²M), addition of EDTA increases the seed germination to 17.6% in onion, 21.4% in radishes, 42.6% in brussels sprout and 77.8% in beans compared to the seed germination in the presence of Pb-acetate alone (Table 2).

Chelating agents in their appropriately concentrations could counteract Pb toxicity, but superfluous resulted in less Pb uptake and growth inhibition of seedlings. Our results demonstrated that EDTA could enhance Pb absorption by the seedlings in both lower and higher contents of Pb. Khodadoust et al. [40] also confirmed that EDTA and organic acid were effective in removing Pb.

Lead concentrations from 10⁻²M to 10⁻⁵M Pb-acetate in the presence of EDTA do not deviate much and are on the level of the seed germination in control variant (only EDTA). In carrot and brussels sprout seeds, a Pb-acetate concentration of 10⁻⁵M in the presence of EDTA decreases seed germination by 2.7% and 2.3% respectively, in relation to the control.

Lead has been reported to strongly affect the seed morphology and physiology [41]. The major effects of heavy metals on seeds are manifested by overall abnormalities and decrease in germination [42], reduced root and shoot elongation, dry weight, total soluble protein level [43], oxidative damage, membrane alteration, altered sugar and protein metabolisms, nutrient loss [44; 16] all contributing to seed toxicity and productivity loss. Pb toxicity has been reported to retard the radical emergence via enhanced pro-

TABLE 2 - The influence of Pb-acetate +EDTA on the seed germination of some vegetable species under experimental intoxications

	beans	onion	raddish	carrot	lettuce	brussels sprout
Control (EDTA)	100.0	100.0±	100.0	100.0	100.0	100.0
10 ⁻⁵ M Pb-acetate +EDTA	99.5±1.0 ^a	100.0±1.0 ^a	100.0±0.6 ^a	103.0±0.4 ^a	100.0±0.5 ^a	102.0±0.7 ^a
10 ⁻⁴ M Pb-acetate +EDTA	98.9±1.5 ^a	99.0±0.8 ^a	99.0±0.9 ^a	96.2±1.0 ^a	94.9±1.6 ^a	100.0±1.0
10 ⁻³ M Pb-acetate +EDTA	98.9±1.2 ^a	98.0±1.2 ^a	98.0±1.3 ^a	95.1±1.6 ^a	91.0±2.0 ^a	94.3±2.5
10 ⁻² M Pb-acetate +EDTA	94.2±2.0 ^a	96.9±1.7 ^a	91.8±2.5 ^a	91.4±1.9 ^a	0.00±0 ^d	81.8±3.5 ^{ab}
2x10 ⁻² M Pb-acetate +EDTA	92.1±2.5 ^a	86.5±2.1 ^{ab}	65.3±3.6 ^b	0.00±0 ^d	0.00±0 ^d	45.5±4.5 ^c

tein and carbohydrate contents, affecting the activity of many enzymes [45]. Pb-polluted soils have been shown to inhibit seedling growth via increased lipid peroxidation, and activation of superoxide dismutase (SOD), guaiacol peroxidase (POD) and ascorbate peroxidase (APX) enzymes [46].

In the majority of species the seed coat constitutes the effective barrier against penetrating of toxic ions [47]. On that account the content of harmful elements in the seed coat is usually two-to four-times higher than in the embryo [48].

If the seed coat is permeable to metals, it can cause delaying or inhibition of germination. As a result, the results presented in this study show that germination was delayed in seeds of *Phaseolus vulgaris* due to exposition to metals, especially lead. Seeds of bean may lose their germinability in different concentrations of metals because it has highly lead permeable seed coats. Studies have shown that seed coats are the main barriers to metals and prevent contamination of embryos [42].

According to Weiqiang et al. [49] tissues covering the embryo are able to protect the embryo against heavy metal toxicity by selective penetration but the following seedling growth (after breakage of seed coat) was virtually repressed at lower concentrations of heavy metals.

We show that lower applied concentrations of Pb (10^{-5}M , 10^{-3}M) did not significantly accumulate and mobilize lead in the endosperm (0.22 and $5.50 \mu\text{g g}^{-1}$) and embryo (5.33 and $20.52 \mu\text{g g}^{-1}$ in medium and large size of seed). At the highest Pb-acetate ($2 \times 10^{-2}\text{M}$) concentration, the content of Pb in the seed coat was the highest ($1139 \mu\text{g g}^{-1}$) in a seed with small total mass, while the lowest accumulation ($1052 \mu\text{g g}^{-1}$)

was obtained in the largest seed. It is possible that accumulation of Pb in the seed coat is a form of protection mechanism from the unfavorable effects of high concentrations and lead accumulation in the central parts of the seed (embryo). The endosperm and embryo show a similar pattern, but at a lower level of accumulation (Table 3).

Seeds with the largest total mass accumulate less content of Pb in embryo and endosperm (Table 4). Small seeds possess a greater surface area to volume ratio than large seeds. This greater surface area permits them to have access to a greater amount of lead than larger seeds, which means they will accumulate more lead. Based on the obtained results, it can be concluded that the bean is characterized by a significant tolerance to Pb contamination during seed germination and growth of seedlings (Table 5). The reason for diminish in seed germination of plant in the presence of high concentration of heavy metals could be due to the accelerated breakdown of stored food materials in seed [50] and or be due changes of selection permeability properties of cell membrane.

Although plant defense strategies exist to cope with heavy metal toxicity via reduced uptake into the cell, sequestration into vacuoles by the formation of complexes, binding by phytochelatins, synthesis of osmolytes, altered expression of enzymes, over expression of genes exist, mechanisms by which germinating seeds combat heavy metal stress remains largely unknown. The future scope of this explorations remains in understanding the biochemistry of heavy metal toxicity in germinating seeds. Understanding such strategies in seeds to overcome such stress and manipulation of pathways and biomolecules involved

TABLE 3 - The effects of total mass of seed bean (*Phaseolus vulgaris*) and concentration of Pb-acetate on accumulation of lead in seed coat

Seed fraction	Pb-acetate concentration		
	10^{-5}M	10^{-3}M	$2 \times 10^{-2}\text{M}$
Small	192.70 ± 16.5^a	767.50 ± 32.5^a	1139.00 ± 43.0^a
Medium	79.10 ± 2.2^b	406.10 ± 23.2^b	1115.00 ± 38.5^a
Large	74.98 ± 1.9^b	82.54 ± 4.2^c	1052.00 ± 32.4^a

TABLE 4 - The effects of total mass of seed bean (*Phaseolus vulgaris*) and concentration of Pb-acetate on accumulation of lead in seed endosperm

Seed fraction	Pb-acetate concentration		
	10^{-5}M	10^{-3}M	$2 \times 10^{-2}\text{M}$
Small	0.16 ± 0.05^a	0.91 ± 0.09^b	580.60 ± 21.30^a
Medium	0.22 ± 0.08^a	5.50 ± 0.18^a	290.20 ± 14.05^b
Large	0.00 ± 0.00^b	0.00 ± 0.00^c	79.45 ± 2.30^c

TABLE 5 - The effects of total mass of seed bean (*Phaseolus vulgaris*) and concentration of Pb-acetate on accumulation of lead in seed embryo

Seed fraction	Pb-acetate concentration		
	10^{-5}M	10^{-3}M	$2 \times 10^{-2}\text{M}$
Small	16.20 ± 1.10^a	110.60 ± 3.50^a	545.50 ± 24.30^a
Medium	5.33 ± 0.30^b	21.70 ± 0.90^b	442.60 ± 19.50^b
Large	2.80 ± 0.09^b	20.52 ± 0.84^b	345.80 ± 14.70^c

will lead to better agricultural produce despite heavy metal toxicity from contaminated soil. The amount of required seed for sowing to achieve the best uniformity and complete land coverage depends, to a great extent, on the type of metal pollutant in germination medium or in irrigation water as well as on their concentrations around the seed.

In conclusion, it can be said that metal salt stress can act on seeds in two ways; first by the prevention of water uptake by seeds and second, by providing conditions for the entry of metal ions that may be toxic to the embryo.

4. CONCLUSION

Increasing concentrations of lead inhibited seed germination and early growth of carrot and lettuce seedlings. Ethylenediaminetetraacetic acid (EDTA) as a chelator reduces the toxicity of lead. The seed coat accumulated significant amounts of Pb and represents a protecting layer for the seed embryo. A greater surface area to volume ratio in small seeds permits them to have access to a greater amount of lead than larger seeds, which means they will accumulate more lead. Based on the obtained results, it can be concluded that bean (especially large seeds) is characterized by significant tolerance to Pb contamination during seed germination and growth of seedlings.

ACKNOWLEDGEMENTS

The first author acknowledges the financial grant from Ministry of Science and Technological Development, Republic of Serbia.

The authors have declared no conflict of interest.

REFERENCES

- [1] Kabala, C., Chodak, T., Szerszen, L., Karczewska, A., Szopka, K. and Frateczak, U. (2009) Factors influencing the concentration of heavy metals in soils of allotment gardens in the city of Wrocław, Poland. *Fresenius Environmental Bulletin* 18: 1118-1124
- [2] Jankiewicz, B. and Adamczyk, D. (2010) Assessing heavy metal content in soils surrounding a power plant. *Polish Journal of Environmental Studies* 19(4): 849-853
- [3] Yargholi, B., Azimi, A. A., Baghvand, A., Liaghat, A. M. and Fardi, G. A. (2008) Investigation of cadmium absorption and accumulation in different parts of some vegetables. *American-Eurasian Journal of Agricultural and Environment Science* 3: 357-364
- [4] Filipović-Trajković, R., Ilić, S. Z. and Šunić, L. (2012) The potential of different plant species for heavy metals accumulation and distribution. *Journal of Food, Agriculture and Environment* 10 (1): 959-964
- [5] Broyer, T. C., Johnson, C. N. and Paull, R. E. (1972) Some aspects of lead in plant nutrition. *Plant Soil* 36: 301-308
- [6] Langerwerff, I. V., Armiger, W. H. and Soecht, A. W. (1973) Uptake of lead alfalfa and corn from soil and air. *Soil Science* 115: 455-460
- [7] Dorogházi, O., Kastori, R. and Maksimović, I. (2010) Nickel translocation from seed during germination and growth of young maize plants. *Proceedings for Natural Sciences published by Matica Srpska - Novi Sad* 119: 17-25
- [8] Lane, S. D. and Martin, E. S. (1977) A histochemical investigation of lead uptake in *Raphanus sativus*. *New Phytologist* 79: 281-286
- [9] Lane, S. D., Martin, E. S. and Garrod, J. F. (1978) Lead toxicity effects on indol-3-acetic acid induced cell elongation. *Planta* 144: 79-91
- [10] Kastori, R., Plesničar, M., Sakač, Z., Panković, D. and Arsenijević-Maksimović, I. (1998) Effect of excess lead on sunflower growth and photosynthesis. *Journal of Plant Nutrition* 21: 75-85
- [11] Wierzbicka, M. and Obidzinska, J. (1998) The effect of lead on seed imbibition and germination in different plant species. *Plan Science* 137: 155-171
- [12] Kastori, R., Petrović, N. and Petrović, M. (1996) Effect of lead on water relations, proline concentration and nitrate reductase activity in sunflower plants. *Acta Agronomica Hungarica* 44: 21-28
- [13] Cseh, E., Fodor, F., Varga, A. and Záray, G. (2000) Effect of lead treatment on the distribution of essential elements in cucumber. *Journal of Plant Nutrition* 23: 1095-1105
- [14] Van Asshe, F. and Clijsters, H. (1990) Effects of metals on enzyme activity in plants. *Plant Cell and Environment* 13: 195-206
- [15] Azmat, R., Haider, S. and Askari, Sh. (2006) Phytotoxicity of Pb: I Effect of Pb on germination, growth, morphology and histomorphology of *Phaseolus mungo* and *Lens culinaris*. *Pakistan Journal of Biological Sciences* 9(5): 979-984
- [16] Pourrut, B., Shahid, M., Dumat, C., Winterton, P. and Pinelli, E. (2011) Lead uptake, toxicity, and detoxification in plants. *Reviews of Environmental Contamination and Toxicology* 213: 113-136
- [17] Wierzbicka, M. (1988) Mitotic disturbance induced by low doses of inorganic lead. *Caryologia* 41: 143-160
- [18] Marques, A.P.G.C., Rangel, A.O.S.S. and Castro, M.L. (2007) Effect of arsenic, lead and zinc on seed germination and plant growth in black nightshade (*Solanum nigrum* L.) vs. clover (*Trifolium incarnatum* L.). *Fresenius Environmental Bulletin* 16(8): 896-903
- [19] Ozdener, Y. and Kutbay, H. G. (2009) Toxicity of copper, cadmium, nickel, lead and zinc on seed germination and seedling growth in *Eruca sativa*. *Fresenius Environmental Bulletin* 18(1): 26-31
- [20] Ling, T. and Jun, R. (2010) Effect of Hg on seed germination, coleoptile growth and root elongation in seven pulses. *Fresenius Environmental Bulletin* 19 (6): 1144-1150
- [21] Jamal, S.N., Iqbal, M.Z. and Athar, M. (2006) Effect of aluminum and chromium on the growth and germination of mesquite (*Prosopis juliflora* (Swartz.) DC). *International Journal of Environmental Science and Technology* 3: 173-176
- [22] Mahmood, S., Hussain, A., Saeed, Z. and Athar, M. (2005) Germination and seedling growth of corn (*Zea-mays* L.) under varying levels of copper and zinc. *International Journal of Environmental Science and Technology* 2: 269-274
- [23] Golubović-Čurguz, V., Raičević, V., Veselinović, M., Tabaković-Tošić, M. and Vilotić, D. (2012) Influence of heavy metals on seed germination and growth of *Picea abies* L. Karst. *Polish Journal of Environmental Studies* 21(2): 355-361

- [24] Mandal S. M. and Bhattacharyya, R. N. (2007) Heavy metal toxicity on seed germination of four pulses. *International Journal of Plant Science* 2(2): 124-127
- [25] Kalimuthu, K. and Siva, S. R. (1990) Physiological effects of heavy metals on *Zea-mays* (maize) seedlings. *Indian Journal of Plant Physiology* 33: 242-244
- [26] Hussain, A., Abbas, N., Arshad, F., Akram, M., Iqbal, Z. K., Ahmad, K., Mansha, M. and Mirzaei, F. (2013) Effects of diverse doses of Lead (Pb) on different growth attributes of *Zea mays* L. *Agricultural Sciences* 4(5): 262-265
- [27] Ilić, Z. and Milanović, L. (1997) The influence of Pb-acetate on the seed germination of some vegetable species under condition of experimental inoxygenation. *Selekcija i Seminarsvo* 4(3-4): 115-119
- [28] Arsenijević-Maksimović, I., Jelić, M., Lomović, S. and Kastori, R. (2001) Genetic and physiological bases of plant tolerance to high concentrations of heavy metals and aluminium. In: Quarrie S et al. (ed.) *Genetic and Breeding of Small Grains*. Agricultural Research Institute Serbia. Beograd, 377-405
- [29] Horbowicz M., Debski H., Wiczowski W., Szawara-Nowak D., Koczkodaj D., Mitrus J. and Sytykiewicz, H. (2013) The impact of short term exposure to Pb and Cd on flavonoid composition and seedling growth of common buckwheat cultivars. *Polish Journal of Environmental Studies* 22(6): 1723-1730
- [30] Paivoke, A.E.A. (2002) Soil lead alters phytase activity and mineral balance of *Pisum sativum*. *Environmental and Experimental Botany* 48: 61-73
- [31] Jiang, W.S. and Liu, D.H. (1999) Effects of Pb²⁺ on root growth, cell division and nucleolus of *Brassica juncea* L. *Israel Journal of Plant Sciences* 47: 153-156
- [32] Thakkar, A. (2014) Analysis of soil lead (Pb) on seed germination, seedling growth and antioxidant activity of gram (*Cicer arietinum*) and pea (*Pisum sativum*). *Asian Journal of Advanced Basic Sciences* 2: 122-124
- [33] Saderi, S.Z. and Zarinkamar, F. (2012) The effect of different Pb and Cd concentrations on seed germination and seedling growth of *Matricaria chamomilla*. *Advances in Environmental Biology* 6: 1940-1943
- [34] Xiong Z.T. (1998) Lead uptake and effects on seed germination and plant growth in a Pb hyperaccumulator *Brassica pekinensis* Rupr. *Bulletin of Environmental Contamination and Toxicology* 60: 285-291
- [35] Szigei, Z. (1998) Növények és a stressz. In: Láng, F. (ed.) *Növényélet – A növényi anyagcsere*. Budapest, ELTE Eötvös Kiadó 915-984
- [36] Wozny, A., Zatorska, B. and Wierzbicka, F. M. (1982) Influence of lead on the development of lupin seedlings and ultrastructural localization of this metal in the root. *Acta Societatis Botanicorum Poloniae* 51: 345-351
- [37] Mukherji, R. and Maitra, P. (1977) Growth and metabolism of germinating rice *Oryza sativa* L.) seeds as influenced by toxic concentrations of lead. *Z. Pflanzenphysiol* 81: 26-33
- [38] Fargasová, A. (1994) Effect of Pb, Cd, Hg, As and Cr on germination and root growth of *Sinapsis alba* seeds. *Bulletin of Environmental Contamination and Toxicology* 52: 452-456
- [39] Filipović, R. and Jablanović, M. (1996) Accumulation and distribution of heavy metals in plant tissues and origins in the location of Kosovska Mitrovica. *University Thought, Natural Science* 3(1): 17-22
- [40] Khodadoust, K.R., Reddy, K.R. and Maturi, K. (2005) Effect of different extraction agents on metal and organic contaminant removal from a field soil. *Journal of Hazardous Materials* 117: 15-24
- [41] Hatamzadeh, A., Sharaf, A.R.N., Vafaei, M.H., Salehi, M. and Ahmadi, G. (2012) Effect of some heavy metals (Fe, Cu and Pb) on seed germination and incipient seedling growth of *Festuca rubra* ssp. *commutata* (Chewings fescue). *International Journal of Agricultural Sciences* 4: 1068-1073
- [42] Kastori, R., Maksimović, I., Doroghazi, O. and Putnik-Delić, M. (2012) Effect of lead contamination of maize seed on its biological properties. *Proceedings for Natural Sciences published by Matica Srpska - Novi Sad* 123: 75-82
- [43] Wang, W., Vinocur, B. and Altman, A. (2003) Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta* 218: 1-14
- [44] Ahmad, M.S. and Ashraf, M. (2011) Essential roles and hazardous effects of nickel in plants. *Reviews of Environmental Contamination and Toxicology* 214: 125-167
- [45] Singh, H.P., Kaur, G., Batish, D.R. and Kohli, R. K. (2011) Lead (Pb)-inhibited radicle emergence in *Brassica campestris* involves alterations in starch-metabolizing enzymes. *Biological Trace Element Research* 144: 1295-1301
- [46] Wang, C., Tian, Y., Wang, X., Geng, J., Jiang, J., Yu, H., et al. (2010) Lead-contaminated soil induced oxidative stress, defense response and its indicative biomarkers in roots of *Vicia faba* seedlings. *Ecotoxicology* 19: 1130-1139
- [47] Muszynska, E., Hanus-Fajerska, E. and Ciarkowska, K. (2013) Evaluation of seed germination ability of native calamine plant species on different substrate. *Polish Journal of Environmental Studies* 22: 1775-170
- [48] Ernst, W.H.O., Verkelyi, A. and Schat, H. (1992) Metal tolerance in plants. *Acta Botanica Neerlandica* 41: 229-248
- [49] Weiqiang, L., Mohammad, A.K., Shinjiro, Y. and Yuji, K. (2005) Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana*. *Plant Growth Regulation* 46: 45-50
- [50] Shafiq, M., Iqbal, M.Z. and Athar, M. (2008) Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala*. *Journal of Applied Sciences and Environmental Management* 12: 61-66

Received: May 02, 2014

Revised: July 07, 2014

Accepted: August 06, 2014

CORRESPONDING AUTHOR

Zoran S. Ilić

Faculty of Agriculture Priština-Lešak
Kopaonička bb
38219 Lešak
SERBIA

Phone: + 381 638014966

E-mail: zoran.ilic63@gmail.com