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Effect of lead on germination and some morphological and physiological parameters of 10-day-old seedlings of various plant species

Wpływ ołowiu na kiełkowanie i niektóre parametry morfologiczne i fizjologiczne w 10-dniowych siewkach różnych gatunków roślin

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Abstract
Among the heavy metals, lead (Pb) is one of the most common environmental pollutants. This study examines the effect of 1 mM lead nitrate $Pb(NO_3)_2$ on the germination index, morphological parameters (root length, shoot length, fresh biomass and tolerance index) and physiological parameters (proline, total chlorophyll and carotenoids) in the leaves of 10-day-old seedlings of various species of crop plants under laboratory conditions. All results, when compared to control, showed Pb adversely affecting the morphological and physiological parameters of the test plants. Among the 12 studied plants, three species (pumpkin, rye and wheat) presented high tolerance to Pb compared to the other test plants. The most sensitive to Pb exposure were radish, barley, tomato and alfalfa.

Streszczenie
Wśród metali ciężkich, ołów (Pb) jest jednym z najczęstszych zanieczyszczeń środowiska. Celem pracy było określenie wpływu 1mM $Pb(NO_3)_2$ – na indeks kiełkowania, a także na kształtowanie się parametrów morfologicznych (długość korzenia, długość pędu, świeżą masę, indeks tolerancji) oraz parametrów fizjologicznych (stężenie proliny, chlorofilu całkowitego i karotenoidów) w liściach 10 dniowych sadzonek różnych gatunków roślin uprawnych w warunkach laboratoryjnych. Ołów niekorzystnie wpływał na parametry morfologiczne i fizjologiczne testowych roślin. Spośród 12 badanych roślin trzy gatunki (dynia, żyto i pszenica) charakteryzowały się wysoką tolerancją na Pb w porównaniu do pozostałych badanych roślin. Najbardziej wrażliwe na działanie ołowiu były rzodkiewka, jęczmień, pomidor i lucerna.

1. INTRODUCTION

Pollution by heavy metals is one of the most important problems worldwide.

Among the heavy metals, lead (Pb) is a highly toxic and persistent environmental pollutant derived from various sources [Sharma and Dubey 2005]. Pb has been extensively used in modern industries to manufacture products such as lead-acid batteries, radiation shields, gasoline, paint, pesticides, ceramics and chemicals. The highest levels of Pb in air are generally found near Pb smelters. Other areas are near industrial effluents, waste incinerators, lead–acid battery manufacturers and while applying phosphate fertilisers [Sharma and Dubey 2005, Shafiq *et al.* 2008, Kabir *et al.* 2010].

Pb is a toxic environmental contaminant that induces many morphological, physiological and biochemical changes in plants. Pb toxicity leads to decreases in the percentage of seed germination [Shafiq *et al.* 2008] and in the growth and yield of plants [Shafiq *et al.* 2008, Kumar and Jayaraman 2014], disruption of mineral nutrition [Lamhamdi *et al.* 2013, Nareshkumar *et al.* 2014], inhibition of photosynthesis [Tian *et al.* 2014], inhibition of enzyme activity [Malar *et al.* 2014], water imbalance and alterations in membrane permeability [Sharma and Dubey 2005, Israr and Sahi 2008]. According to Wang [1987], germination and root growth are stages in plant development that are especially sensitive to contamination. Therefore, these stages may be observed for a fast biological assessment of environmental contamination as well as to make a preliminary assessment, selection and characterisation of a contamination-resistant species of crop plants [Wang and Williams 1988]. Cultivation of varieties that are tolerant to various environmental stressors is the easiest and cheapest way to counteract crop losses caused by them [Ashraf and Harris 2005]. Selecting cultivars tolerant to abiotic stresses contribute to improving crop yields.

The aim of this study is to determine the effect of 1 mM lead nitrate - $Pb(NO_3)_2$ on the morphological and physiological parameters of the leaves of 10-day-old seedlings of various species of crop plants, and assessment of sensitivity to the presence of Pb ions in the environment, which is the basis for the selection of certain plant species tolerant to unfavourable environmental conditions.

2. MATERIALS AND METHODS

The experiment was conducted in January 2014 in the laboratory of the Department of Plant Physiology and Biochemistry and also Department of Plant Genetics, Breeding and Biotechnology at the West Pomeranian University of Technology in Szczecin, Poland. The study material included undressed seeds collected from 12 plant species commonly used in testing the toxicity (phytotoxicity): pumpkin (*Cucurbita pepo,* var. 'Danka Polka'), radish (*Raphanus dativus,* var. 'Carmen'), cucumber (*Cucumis sativus* L.), barley (*Hordeum vulgare* var. 'Eunova'), rye ordinary (*Secale cereale* var. 'Bojko'), wheat (*Triticum aestivum* L. var. 'Bryza'), blue lupine (*Lupinus angustifolius* L. var. 'Karo'), sunflower *(Helianthus annuus),* tomato (*Lycopersicon esculentum* var. 'Faworyt'), alfalfa (*Medicago sativa* L.), cuckooflower (*Lepidium sativum*), lentil (*Lens culinaris* Medik.).

Seeds of each species were characterised towards Pb-induced phytotoxicity — lead nitrate [$Pb(NO_3)_2$]. Seeds were surface-sterilised with 70% (v/v) ethanol solution for 30 seconds and then thoroughly rinsed with sterile water. After the preliminary disinfection, the seeds were soaked for 15 minutes in 10% (v/v) solution of sodium hypochlorite (NaOCl), after rinsing three times in sterile water. Next, seeds were placed in petri dishes lined (Ø10 cm) with filter paper and moistened with 30.0 cm^3 sterile water (control) and of Pb solution. The experiment was established in three replications of 10 seeds in duplicate. The experiments were repeated three times.

The plates were incubated at 21°C in the dark for 72 hours, then the germinated seeds were counted and the root length measured. With both values, calculated Index of Germination (IG%) from the formula given by Barbero *et al*. [2001]:

$$IG\ \% = (GS\ Ls)/(Gc\ Lc) \times 100$$

where:

Gs and Ls - the seed germination and root length (mm) of the plant exposed to stress Pb;

Gc and Lc - the corresponding values for controls.

Then, the test plants were transferred to phytotron at the Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology in Szczecin, Poland and kept in highly regulated thermal (25°C), humidity (70–80%) and light (around 40 $\mu E \cdot m^{-2} \cdot s^{-1}$) conditions. During the study a 16:8 hour photoperiod was maintained.

After 10 days of growth, the effect of 1 mM $Pb(NO_3)_2$ on the morphological parameters (root length, shoot length, fresh biomass and tolerance index) and physiological parameters (proline, total chlorophyll and carotenoids) of 10-day-old seedlings of various species of crop plants were determined.

The *tolerance index (TI)* The tolerance index (TI) was calculated by dividing the root length of the plant exposed to stress Pb by that measured during growth in the control solution. The following equation was used:

TI (%) = 100 x (root length under Pb treatment)/(root length in the control solution).

Determination of proline Proline content was measured according to the method of Bates *et al*. [1973]. Fresh seedlings (0.5 g) were ground in 1.5 ml of aqueous sulfosalicylic acid 3% (w/v), and proline was estimated by ninhydrin reagent. The samples extracted with toluene and the absorbance of the toluene phase was read at 520 nm. The concentration of proline was calculated from a standard curve and expressed as $\mu mol \cdot g^{-1}$ fresh weight.

Determination of pigments The extraction of leaf pigments was performed with 80% (v/v) acetone. Chlorophyll a, b and carotenoids content was determined spectrophotometrically at 663, 645 and 440 nm. The concentration of total chlorophyll were calculated according to the method of Arnon *et al*. [1956] in modification to Lichtenthaler and Wellburn [1983], whereas the concentration of carotenoids was calculated according to the method of Hager and Meyer-Berthenratha [1966]. The pigment concentrations were expressed as $\mu g \cdot g^{-1}$ fresh weight.

Statistical analysis The significance of differences was determined by means of variance analysis and Tukey's test, at the level of significance of $\alpha = 0.05$.

3. RESULTS AND DISSCUSION

Growth inhibition is a common response of plants to heavy metal stress and is also one of the most important agricultural indices of heavy metal tolerance [Jiang and Liu 2010]. Pb toxicity has become important because of the steadily increasing levels of this metal in the environment. The effect of Pb on seedling growth seems to be different with regard to plant species, cultivars, organs and metabolic processes [Sharma and Dubey 2005].

Pb treatment showed toxic effect on seed germination, root growth, seedling growth and dry biomass of plant species tested with respect to control (Figure 1 and table 1). The test plants had various index of germination (Fig. 1). The values of index of germination were the highest for cucumber (40.40%), and the lowest for sunflower (9.20%).

Seed germination inhibitions by heavy metals have been reported by some researchers [Mahmood *et al*. 2005, Jamal *et al*. 2006]. These decreases in germination may be due to the interference of Pb with metabolic processes, which loss of viability decrease of energy generation for on embryo. Energy generation is very important for seed germination and its blockage affects protein, nucleic acids production, as well as mitosis [John and van-Laerhoven 1976].

The application of lead nitrate had a significant effect on decrease ($p \le 0.05$) of root length in the studied plant species compared to the control plants. The highest decrease of root length was found in sunflower (90.14%), tomato (89.74%), alfalfa (84.76%) and lentil (81.16%) (Table. 1). The least sensitive to Pb was pumpkin, rye and wheat. In case of pumpkin and rye, no statistically significant difference was observed among root lengths from comparable culture conditions.

Evaluating seedling length in the studied plant species, it was found that the presence of Pb resulted in decrease in seedling length in the test plants compared to the control plants. The greatest reduction in seedling length was observed in alfalfa (60.89%), sunflower (45.43%) and parsley (44.19%) (Table 1). On the other

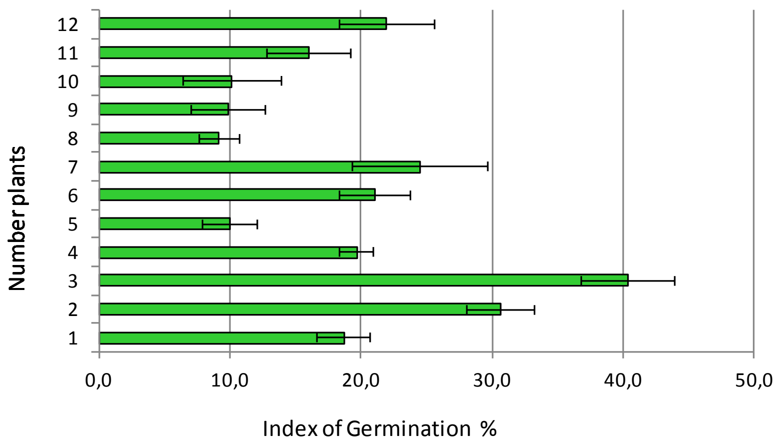

Figure 1. The value of IG% of seedlings of various species of plants growing under stress conditions induced with 1 mM Pb salt. The error bars indicate mean ± SD (n = 3); Nambers 1–12 are: 1 - pumpkin, 2 - radish, 3 - cucumber, 4 - barley, 5 - rye ordinary, 6 - wheat, 7 - blue lupine, 8 - sunflower, 9 - tomato, 10 - alfalfa, 11 - cuckooflower and 12 - lentil; IG%: index of germination.

Table. 1. Summary of morphological features and the fresh weight of seedlings of different species of plants growing under conditions of stress induced with 1 mM Pb salt

Plant species	Root length [cm]			Shoot length [cm]			Fresh weight [g]			Tolerance index [%])
	Control	1 mM soli Pb	$LSD_{0.05}$	Control	1 mM soli Pb	$LSD_{0.05}$	Control	1 mM soli Pb	$LSD_{0.05}$	
Pumpkin (*Cucurbita pepo*, var. 'Danka Polka')	6.53 ± 1.32 (100)	5.67 ± 1.25 (86.83)	n.d.	4.96 ± 0.65 (100)	4.7 ± 1.47 (94.76)	n.d.	1.4 ± 0.35 (100)	1.2 ± 0.06 (85.71)	n.d.	86.09
Radish (*Raphanus dativus*, var. 'Carmen')	3.22 ± 1.74 (100)	0.99 ± 0.34 (30.75)	1.39*	2.51 ± 1.12 (100)	1.75 ± 0.614 (69.72)	0.62*	0.07 ± 0.03 (100)	0,08 ± 0,04 (114,29)	n.d.	30.75
Cucumber (*Cucumis sativus* L.)	8.51 ± 2.53 (100)	3.44 ± 2,41 (40.42)	2.91*	2,17 ± 0,51 (100)	1,62 ± 0,50 (74,65)	0.41*	0.12 ± 0.07 (100)	0,11 ± 0,06 (91,67)	0.05*	40.42
Barley (*Hordeum vulgare* var. 'Eunova')	8.23 ± 1.61 (100)	1.83 ± 0.92 (22.24)	1.29*	9.98 ± 1.6 (100)	6.84 ± 0.64 (68.54)	0.84*	0.24 ± 0.06 (100)	0.16 ± 0.03 (66.67)	0.03*	22.24
Rye ordinary (*Secale cereale* var. 'Bojko')	8.58 ± 2.32 (100)	5.90 ± 2.32 (68.76)	n.d.	7.46 ± 2.31 (100)	4.84 ± 1.35 (64.88)	1.95*	0.17 ± 0.07 (100)	0.13 ± 0.05 (76.47)	0.04*	68.76
Wheat (*Triticum aestivum* L. var. 'Bryza')	6.23 ± 1.19 (100)	3.74 ± 1.13 (60.03)	2.28*	13.07 ± 2.98 (100)	7.25 ± 2.20 (55.47)	1.67*	0.19 ± 0.04 (100)	0.13 ± 0.03 (68.42)	0.02*	60.03
Blue lupine (*Lupinus angustifolius* L. var. 'Karo')	2.66 ± 0.63 (100)	0.71 ± 0.34 (26.69)	0.48*	6.05 ± 1.76 (100)	3.75 ± 1.06 (61.98)	0.94*	0.57 ± 0.17 (100)	0.46 ± 0.12 (80.70)	0.09*	26.69
Sunflower seeds (*Helianthus annuus*)	8.01 ± 3.11 (100)	0.79 ± 0.13 (9.86)	2.01*	4.05 ± 1.18 (100)	2.21 ± 0.85 (54.57)	0.76*	0.46 ± 0.18 (100)	0.22 ± 0.07 (47.83)	0.08*	9.86
Tomato (*Lycopersicon esculentum* var. 'Faworyt')	12.48 ± 3.05 (100)	1.28 ± 0.37 (10.26)	1.43*	2.25 ± 0.44 (100)	1.43 ± 0.40 (63.56)	0.22*	0.1 ± 0.04 (100)	0.09 ± 0.05 (90.00)	n.d.	10.26
Alfalfa (*Medicago sativa* L.)	4.33 ± 0.92 (100)	0.66 ± 0.25 (15.24)	1.58*	2.71 ± 0.79 (100)	1.06 ± 0.55 (39.11)	0.68*	0.02 ± 0.01 (100)	0.01 ± 0.005 (50.00)	n.d.	15.24
Cuckooflower (*Lepidium sativum*)	11.75 ± 2.30 (100)	2.67 ± 0.68 (22.72)	2.72*	2.62 ± 0.73 (100)	2.41 ± 0.74 (91.98)	n.d.	0.03 ± 0.02 (100)	0.01 ± 0.01 (33.33)	0.01*	22.72
Lentil (*Lens culinaris* Medik.)	3.29 ± 1.81 (100)	0.62 ± 0.24 (18,84)	0.94*	2.13 ± 0.66 (100)	2.18 ± 0.68 (102.35)	n.d.	0.12 ± 0.05 (100)	0.07 ± 0.04 (58.33)	0.03*	18.84

$LSD_{0.05}$– less significant difference *a* < 0.05; ± SD – standard deviation; * The significance of differences at the level of *a* <0.05; n.d.- nonsignificant difference;

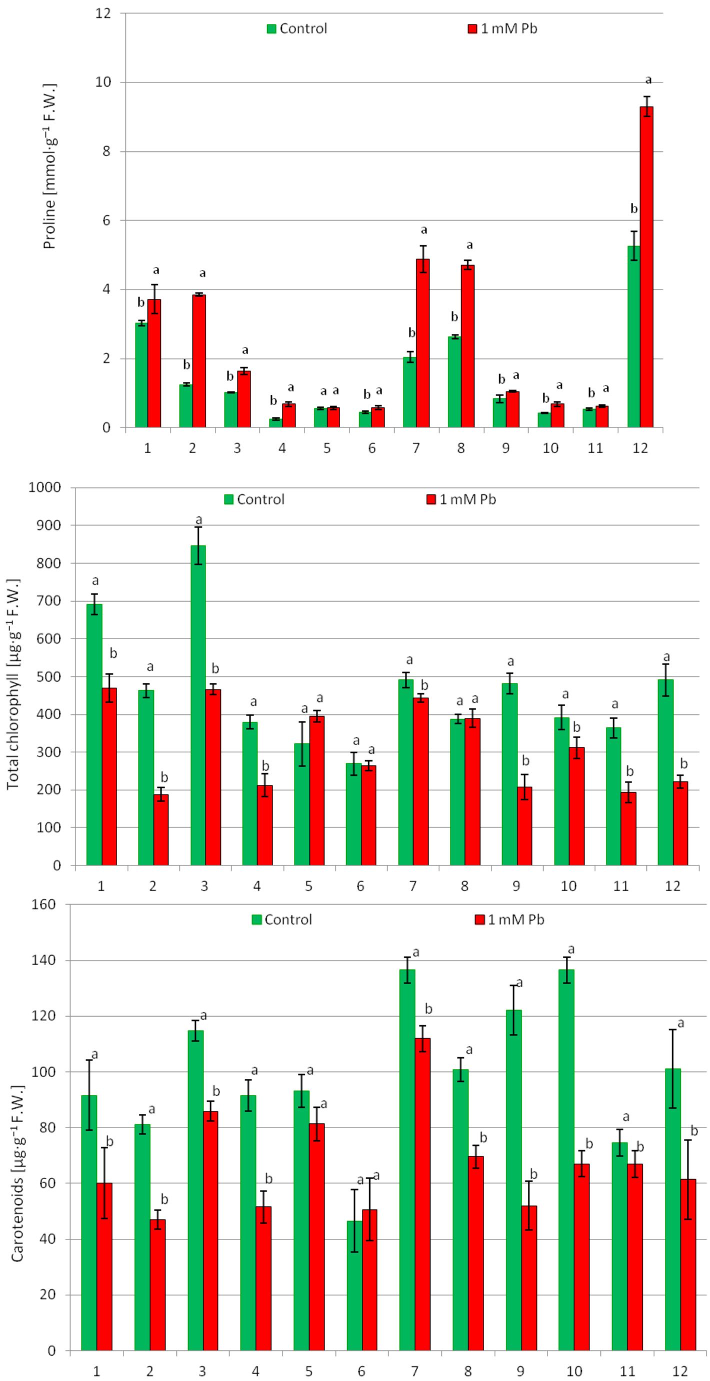

Figure 2. Summary of physiological parameters in the leaves of seedlings of various species of plants growing under stress conditions induced with 1 mM Pb salt. The error bars indicate mean ± SD (n = 3), followed by different letter are statistically significant at $\alpha < 0.05$ levels. F.W. - fresh weight; Numbers 1–12 are: 1 -pumpkin,2 - radish, 3 - cucumber, 4 - barley, 5 - rye ordinary, 6 - wheat, 7 - blue lupine, 8 - sunflower*,* 9 - tomato, 10 - alfalfa, 11 - cuckooflower and 12 - lentil;

side, in pumpkin, radish, cress and lentil, no statistically significant difference was observed in the seedling growth of plants originating from control and control with 1 mM Pb. The inhibitory effect was more pronounced on root length than on shoot length (Table 1). Sharma and Dubey [2005] reported that roots assimilate Pb better than leaves; therefore, symptoms of toxicity are more enhanced in underground than overground organs. This dependence was also confirmed by Grzesiuk *et al*., [2011], who studied sensitivity to Pb of two varieties of buckwheat. Inhibition of root growth and seedling to Pb was previously noticed in various plants [Shafiq *et al*. 2008, Wang *et al*. 2011, Malar *et al*. 2014]. But, Grzesiuk *et al*. [2011] found that low concentration of Pb (0.01 mM) in roots had a stimulatory effect on their growth; although, reduction of their growth by 60% occurred at 1 mM of Pb. Reduced growth of plants could be related to lower number of cell divisions in zone of cell division [Eun *et al*. 2000]. Whereas Jiang and Liu [2010] observed that exposure of *Allium sativum* roots to Pb for 72 hours induced ultrastructural changes, that is: loss of cristae, mitochondrial swelling, dictyosomes, endoplasmic reticulum vacuolisation and impairment into lamellar organisation of the chloroplast.

In our studies, a significant decrease in fresh biomass ($p \leq 0.05$) was observed in plants growing with concentration of Pb ranging from 8.4 to 66.7% compared to the control (Table 1). Similar decrease in fresh biomass was also found in stress conditions caused by Pb in *Chlorophytum comosum* [Wang *et al*. 2011] and *Eichhornia crassipes* [Malar *et al*. 2014].

TI is an important indicator that reflects the heavy metal tolerance of plants [Yan *et al*. 1997]. After 10 days of exposure to Pb, the TI value in the studied plant species was low that means about high sensitivity of the test plants to Pb (Table 1). The only plants were TI values were higher were recorded in pumpkin, rye and wheat and they were 86.06, 68.76 and 60.03%, respectively. In studies carried out by Shaikh *et al*. [2013], TI was decreasing along with the increasing concentrations of Cr, Cd, Mn and Zn in wheat.

With the absorption and accumulation of heavy metal, the plant undergoes many physiological changes [Liu *et al*. 2009].

Literature data shows that proline has a positive impact to plant water management as well a pivotal role in plant response to metal presence, which is probably related to antioxidative properties, role of chelating metals and ability of proline to protect enzymes [Özturk and Demir 2002]. In our study, concentration of proline significantly increased in the test plants that obtained a dose of Pb at a concentration ranging from 25 to 208% (Figure 2). Similar response to Pb treatment was previously noticed in various plants [Zengin and Munzuroglu 2005, Awaad *et al*. 2010].

Chlorosis of leaves is one of the physiological symptoms of Pb action on plants. Chlorosis is caused by inhibition of synthesis of photosynthetic pigments [Sharma and Dubey 2005]. Myśliwa-Kurdziel and Strzałka [2002] reported that δ-aminolevulinic acid dehydratase, which is involved in the chlorophyll biosynthetic pathway, is very sensitive to the presence of heavy metals.

Pb treatments showed a statistically significant decrease ($p \leq 0.05$) on photosynthetic pigments - total chlorophyll content and carotenoids of plants leaves compared to control (Figure 2).

In studies by Malar *et al*. [2014], where plants were treated to 1000 mg·L^{-1} of Pb, the reduction in chlorophyll a, b and carotenoid contents was 55, 67 and 55%, respectively, compared to the control.

4. CONCLUSIONS

Based on the obtained results, it was assumed that the concentration of Pb applied in this study inhibited seed germination and growth of roots and shoots, fresh biomass of plants and the content of assimilation pigments and increased the content of proline in 10-day-old seedlings in the studied plant species.

However, reaction of the test plants to Pb was different. Within the studied plants, there were species that in lower degree reacted to stress conditions. In these plants, small or no statistically significant difference was found in morphological and physiological parameters between the control plants and plants exposed to Pb. Among the 12 studied plants, 3 species (pumpkin, rye and whet) presented high tolerance to Pb.

Among the studied plants, there were also species that significantly reacted to stress conditions. This reaction was reflected by statistical significant decrease in average morphological and physiological parameters: decrease in the content of assimilation pigments and increase in proline level compared to the control plants. The most sensitive to Pb exposure were radish, barley, tomato and alfalfa.

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