Annales Universitatis Paedagogicae Cracoviensis
Studia Naturae, 6: 63–80, 2021, ISSN 2543-8832
DOI: 10.24917/25438832.6.4

Elke Bloem[1*], Jowita Ciaranek[2]

[1]Institute for Crop and Soil Science, Federal Research Centre for Cultivated Plants (JKI),
Bundesallee 69, 38116 Braunschweig, *elke.bloem@julius-kuehn.de

[2]Institute of Biology, Pedagogical University of Krakow, Podchorążych 2 St., 30-084 Kraków, Poland

The influence of lead compounds on selected morphological features and the physiological processes of *Zea mays* L.

Introduction

Soil is a valuable, non-renewable environmental resource, essential for the development and continuity of plant growth and life. The soil stores, filters and transforms various substances, including nutrients and carbon. It contains mineral salts and water – substances necessary for the seed germination process, for the growth and development of most plants. It is a heterogeneous mixture of organic and inorganic compounds with various particle sizes (Kabata-Pendias, Pendias, 2001).

Soils should be protected because of their importance, both for the natural environment and in the economic and social context. However, in the modern world, soil degradation is a serious problem. The reason for this degradation is anthropopression manifested by: inadequately conducted agricultural and forestry works, industrial activity, tourism, uncontrolled development of cities and industrial regions, and improper land development (Marcinek et al., 1995; Ettler, 2016; Możdżeń et al., 2017; Huo et al., 2020). As side effect of the process of industrialisation, urbanisation and technology advances, one of the many problems around the world is the release of heavy metals into the soil. Heavy metal pollution causes adverse changes in soil properties, has a direct impact on water quality, and negatively affects biodiversity and climate change (Rodriguesa et al., 2017). Such diffuse pollution also threatens the production of organic food (Konieczna et al., 2018a, b).

Among heavy metals, lead (Pb) is an element that accumulates easily in soils and sediments (Kabata-Pendias, Pendias, 2001). The main sources of its pollution are emissions from copper, iron and steel smelters, smelted zinc ores and cement production (Pattee, Pain, 2002; Carocci et al., 2016). In many countries around the world, the release of lead has been reduced, but unfortunately it is still used in the automotive industry,

in the production and recycling of batteries, boat building, pottery and book printing, ammunition for hunting. Due to its non-biodegradable nature, its concentration accumulates in the environment, which contributes to an increase in the threat to health and life of organisms (Jiao et al., 2012; Li et al., 2012; Arnemo et al., 2016; Zhou et al., 2018).

Although lead is not an essential element for plants, it is easily absorbed and accumulates in different plant parts. The highest amounts of lead were found in the roots of plants, with the simultaneous limitation of its translocation to the above-ground parts (Seregin, Ivanov, 2001). Lead uptake by plants is regulated by the pH, particle size and cation exchange capacity of the soil. The excess of lead causes blackening of the root system of plants, growth inhibition and chlorosis. Moreover, photosynthesis is disturbed, mineral nutrition and water balance are disrupted, the hormonal status is changed and the structure and permeability of cell membranes is affected (Sharma, Dubey, 2005; Puła et al., 2019). The toxic effect of lead on living organisms is mainly related to the reaction of lead ions with sulfhydryl groups of enzymes and proteins (Kabata-Pendias, Pendias, 2001; Kabata-Pendias, Mukherjee, 2007). The extent to which this metal affects plants varies with its concentration as well as with plant species. Some plants can tolerate levels of heavy metals that are already toxic to other plants (Kranner, Colville, 2011).

Although studies of the lead-plant interactions are quite frequent, few experiments have been performed simultaneously at the stage of seed germination and plant growth. Therefore, the aim of the current study was to determine the germination of grains and the growth of maize (*Zea mays* L.) plants that are equally exposed to lead in the form of nitrate compounds. It was hypothesised that the percentages of the lead solution used had a different effect on the physiological parameters of maize. Therefore, analyses were carried out on (*1*) grains germination capacity, (*2*) plant growth, (*3*) fresh and dry mass of maize organs and (*4*) photosynthetic activity by measuring photosynthesis and transpiration.

Material and methods

Plant material

Grain of maize (*Zea mays* L.) was purchased in the POLAN garden store. These grains were used in Petri dishes germination biotests and for cultivation in the growth chamber.

Solution used in experiment

Water solutions of $Pb(NO_3)_2 \times 4H_2O$ were prepared according to the procedure for 0.1%, 1% and 3% solutions. A solution containing 1% was prepared from 1 g of a chemical compound dissolved in 99 ml of distilled water. The other solutions were prepared accordingly and stored in the refrigerator for the duration of the experiment.

Germination condition

Maize grains (*Zea mays* L.), rinsed under running distilled water by 30 min., were placed in sterile Petri dishes with a diameter of 15 cm with three layers of filter paper (100 grains per Petri dish). Each Petri dish was soaked with 10 ml of the appropriate solution and 3 ml was watered every other day. The control consisted of Petri dishes with grains watered with distilled water in the same amounts as the Petri dishes with lead solutions. The germination test in Petri dishes was stored in the dark at a temperature of 25°C (day / night) and under a relative air humidity of 60–70%. After 24 hours, the germinated grains were counted. The operation was repeated after 48 h, 72 h and 96 h.

Plant cultivation

Maize grains germinated in Petri dishes were planted in 0.5 litre pots with sand and were transferred into a growth chamber with a light intensity of 200 $\mu mol \times m^{-2} \times s^{-1}$ (LI-COR, USA), during the photoperiod of 12 h and with a temperature of 25°C and a temperature of 20°C. The relative humidity (RH) was 70–80%.

Two different experimental setup were established. In one group of pots plants were grown from grains which germinated already on substrates containing lead(II) nitrate solution and which were watered during their further growth with distilled water. The second group of pots included plants grown from grains germinated in distilled water but which were watered during their growth with lead(II) nitrate solution. The control group consisted of plants, which were watered with distilled water, both during germination and further growth. Once a week, all plants were watered with Steiner medium (Steiner, 1961).

Biometric analysis

The effect of different concentrations of $Pb(NO_3)_2$ solutions on the growth of maize plants was measured with a calliper, with an accuracy of ±0.1 cm. The length of the roots, blades, leaves (first to fifth leaf) and the remaining part of the shoot was measured. Based on the formula of Mominul Islam et al. (2012), the length of the organs expressed as a percentage of control organ was determined (IP):

$$IP\ [\%] = [1 - (L_S / L_C)] \times 100$$

IP – inhibition percentage [%], L_S – organ length [cm] treated with the water solution type, L_C – organ length [cm] treated with the distilled water (control group)

Plant biomass

The fresh mass (FM) of the maize organs was determined on a laboratory balance (Ohaus Adventurer Pro, USA) with an accuracy of 0.0001 g. In order to determine the

dry mass, plant organs were dried in a laboratory dryer (WAMED SUP 100, Poland) for 48 h at a temperature of 105°C. Based on the masses obtained, the percentage of water content was determined according to the formula: WC = [(FM – DM) / FM] × 100 and percentage of dry mass: DM = (DM / FM) × 100; where WC – water content, FM – fresh mass, DM – dry mass.

Photosynthesis and transpiration

The intensity of photosynthesis (P_N) and transpiration (E) was determined for the third maize leaf. For this purpose, the CIRAS-2 infrared gas analyser was used. A PLC 4 board chamber with an area of 2.5 cm^2 was used for measurements. The intensity of light reaching the leaf during photosynthesis measurements was 200 $\mu mol \times m^{-2} \times s^{-1}$, 60–70% humidity, and temperature was about 25°C.

Statistical analysis

The significance of differences between the subjects was tested by Duncan's test for homogeneous groups, at the level of $p \leq 0.05$; mean values ($n = 5, \pm SD$) marked with different letters in rows or columns differ significantly. The calculations were made in Excel and Statistica for Windows 13.1. StatSoft, Inc. (2021).

Results

Germination of maize was affected by the higher concentrated lead solutions (Tab. 1). After 48 h of germination of *Zea mays* L. grains it was observed that grains germinated to a higher extent in the lead salt solution containing 0.1% $Pb(NO_3)_2$, compared to the control and the higher concentrations of lead solutions. The lowest percentage of germinated grains was found on the substrates containing 3% $Pb(NO_3)_2$.

On the third day of germination, the number of germinated grains was lower in each concentration of lead solutions, compared to the germination rate in the control Petri dishes. The significantly, lowest number of seeds germinated in the Petri dishes containing the 3% lead solution. In the next two days of germination, the percentage of germinated grains was the highest in the control, in relation to the lead salt solution used. Along with the increase in the concentration of the solution, a significant inhibition of the germination of maize grains was demonstrated (Tab. 1).

Maize plants developed very differently in relation to the applied lead treatment when grown in pots (Tab. 2). Plants grown from grains germinated already on lead water solutions developed significantly shorter roots compared to plants that germinated without lead but were watered with metal solutions during their growth after germination (Fig. 1; Tab. 2).

Tab. 1. Percentage of germinated maize grains (*Zea mays* L.) treated with solutions of $Pb(NO_3)_2$ at concentrations of 0.1%, 1% and 3% in the germination test in Petri dishes

Time [h]	Control	0.1%	1%	3%
48	56 b	66 a	22 e	2 f
72	92 a	82 b	36 d	12 f
96	94 a	90 b	66 d	20 e
120	99 a	90 b	67 d	21 f

mean values (n = 5) marked with different letters (within the row) differ significantly according to Duncan's test at $p \leq 0.05$

Tab. 2. Development of different plant organs of *Zea mays* L. in length [cm] in relation to lead application at different concentrations during germination in comparison to treatment after germination (Control – seeds and plants treated with distilled water; lead application: $Pb(NO_3)_2$ water solutions at 0.1%, 1% and 3% concentrations during the germination stage or as irrigation during growth)

Organ	Control	Lead application during germination			Lead application during growth		
		0.1%	1%	3%	0.1%	1%	3%
Root	20.80 c	19.82 d	18.32 de	19.70 d	23.80 a	21.40 bc	14.60 g
Blade	6.40 a	3.54 e	3.14 g	4.80 d	5.60 bc	5.70 b	3.30 f
I Leaf	5.60 a	4.52 c	3.42 e	4.80 b	5.30 ab	5.50 ab	4.80 b
II Leaf	15.80 a	10.06 de	9.04 e	12.50 cd	15.20 ab	15.30 ab	13.10 c
III Leaf	23.30 d	18.10 f	17.36 g	21.60 d	26.50 b	28.20 a	18.40 e
IV Leaf	13.60 cd	19.56 b	19.05 b	14.50 c	19.70 b	21.70 a	11.40 e
V Leaf	9.00 a	7.14 b	6.28 c	0.00 h	2.80 f	6.00 c	0.00 h
Rps	5.26 a	5.02 ab	5.24 a	3.00 c	3.60 c	4.10 b	3.40 c

I Leaf, II Leaf, III Leaf, IV Leaf, V Leaf – leaf number, Rps – Remaining part of the shoot; mean values (n = 5) marked with different letters (within the row) differ significantly according to the Duncan test at $p \leq 0.05$

Compared to the control, the roots of *Z. mays* were the longest in plants grown from grains germinated in distilled water and watered with 0.1% $Pb(NO_3)_2$ during growth, and the shortest in plants treated with 3% $Pb(NO_3)_2$. Biometric analyses of blades and leaf I and II showed that each of the solutions inhibited the growth of these organs, regardless of the stage in which lead was applied. In case of the third leaf shorter leaves in comparison to the control were developed in plants which germinated in lead solutions, but longer ones when lead application occurred during growth. The IV leaf was longer in lead-treated plants regardless of the application phase. Compared to the control shorter leaves were observed only in the plants that received 3% $Pb(NO_3)_2$ during growth. The length of fifth maize leaf was the longest for the control plants. Plants germinated in lead solution or watered with 3% $Pb(NO_3)_2$ solution did not develop a fifth leaf. The remaining part of the shoot was significantly the longest in the control plants, compared to the plants receiving lead at germination or during growth (Tab. 2).

The percentage of growth inhibition (IP value) for the different plant organs in relation to lead application is shown in figure 1. The IP clearly indicate that the strongest negative effect was observed for the length of the fifth leaf.

Biomass development of maize in relation to lead application is shown in table 3. The fresh mass of root of the maize plants varied depending on the concentration of the solution.

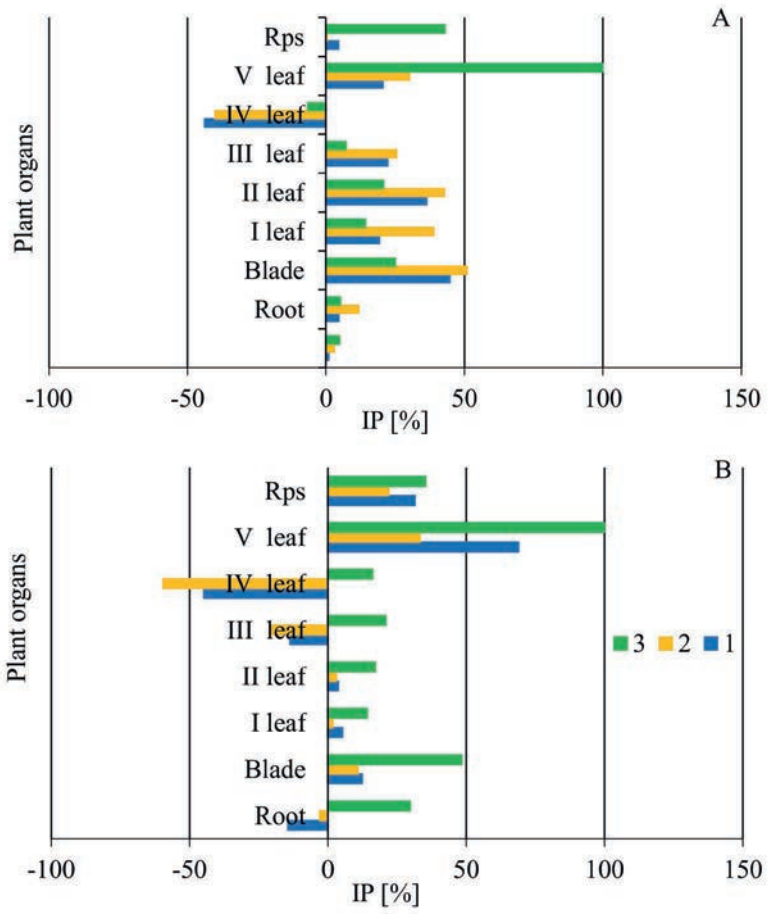

Fig. 1. Length, expressed as the IP (Inhibition Percentage) index in percentage of the control group, of the individual plant organs of *Zea mays* L. (Treatments: Control – watered with distilled water in comparison to $Pb(NO_3)_2$ solutions at concentrations 0.1%, 1% and 3% applied at (A) – germination or (B) – during growth; mean values (n = 5), positive values indicate a negative effect, negative values indicate a positive effect; 1 (blue): 0.1% $Pb(NO_3)_2$, 2 (yellow): 1% $Pb(NO_3)_2$, 3 (green): 3% $Pb(NO_3)_2$; Rps – Remaining part of the shoot

Tab. 3. Development of fresh biomass [g] of individual plant organs of *Zea mays* L. in relation to lead application at different concentrations during germination in comparison to treatment after germination (Control – seeds and plants treated with distilled water; lead application: $Pb(NO_3)_2$ water solutions at 0.1%, 1% and 3% concentrations during the germination stage or as irrigation during growth)

Organ	Control	Lead application during germination			Lead application during growth		
		0.1%	1%	3%	0.1%	1%	3%
Root	2.0122 ab	2.1154 a	1.9886 b	1.7870 c	2.1014 a	1.7925 c	0.7218 f
Blade	0.4916 d	0.4830 d	0.4022 e	0.4906 d	0.7736 b	0.8453 a	0.2836 f
I Leaf	0.0440 f	0.0710 d	0.0640 e	0.0886 cd	0.1210 a	0.1128 b	0.0190 g
II Leaf	0.1518 e	0.1550 e	0.1536 e	0.2216 d	0.3054 a	0.2948 ab	0.1116 g
III Leaf	0.3014 ef	0.3111 e	0.3077 f	0.3700 d	0.5555 b	0.6220 a	0.2082 h
IV Leaf	0.3646 b	0.3706 b	0.3506 b	0.1950 d	0.3934 a	0.4320 a	0.1108 f
V Leaf	0.0924 a	0.0754 b	0.0600 c	0 h	0.0334 f	0.0428 e	0 h
Rps	0.4004 b	0.4298 ab	0.4320 ab	0.2030 d	0.3558 c	0.4123 ab	0.1452 f

I Leaf, II Leaf, III Leaf, IV Leaf, V Leaf – leaf number, Rps – Remaining part of the shoot; mean values (n = 5) marked with different letters (within the row) differ significantly according to the Duncan test at $p \leq 0.05$

The lowest biomass development was recorded for all investigated plant parts in plants treated with solutions containing 3% of lead, compared to the control, regardless of the application stage. The values of the fresh mass of the blades were clearly higher for plants treated with lead solutions during growth in comparison to application at germination stage. Compared to the control, the fresh mass of the first three leaves was higher in plants watered with lead solutions during growth while that of the plants that received lead during the germination stage was comparable. The fourth leaf achieved the highest fresh mass in maize plants watered during growth with 0.1% or 1% $Pb(NO_3)_2$, relative to the control. There were no differences in the values of this parameter between the control plants and the plants germinating in 0.1% and 1% of $Pb(NO_3)_2$. Biomass of the fifth leaf was lower in all treatments in comparison to the control. The fresh mass of the remaining part of the maize shoot was greater in plants grown from grains watered with lead solutions during the germination stage. Generally, in the remaining cases, these values were lower than the control. The lowest fresh mass values were recorded in plants watered with 3% $Pb(NO_3)_2$ during growth.

The percentage of dry mass in the roots of *Z. mays* grown from grains watered with lead solutions was the highest in the control, in relation to all solutions used (Fig. 2). In the case of plants treated with lead solutions during growth, the highest percentage of root dry mass was found in those watered with 3% $Pb(NO_3)_2$. The percentage of dry mass of the blade was lower in each of the lead solutions, in relation to the control, regardless of the watering stage.

Exceptions were noted for plants from the germination stage treated with 0.1% $Pb(NO_3)_2$ and in the growth stage treated with 3% $Pb(NO_3)_2$. For the first maize leaf, lower values of this parameter were found, compared to the values from the control plants. The percentage of dry mass for the 2nd and 3rd leaves differed from the control and depended on the concentration and time of application. In the case of the fifth leaf, lower values of this parameter were observed compared to the control sample. Dry mass for the remaining part of the shoot showed different values compared to the control.

The dry mass of *Z. mays* roots was greatest in the control plants (Tab. 4). Regardless of the treatment stage, a decrease in the mass gain of this organ was observed once the concentration of lead solutions increased. The dry mass values for the blade varied

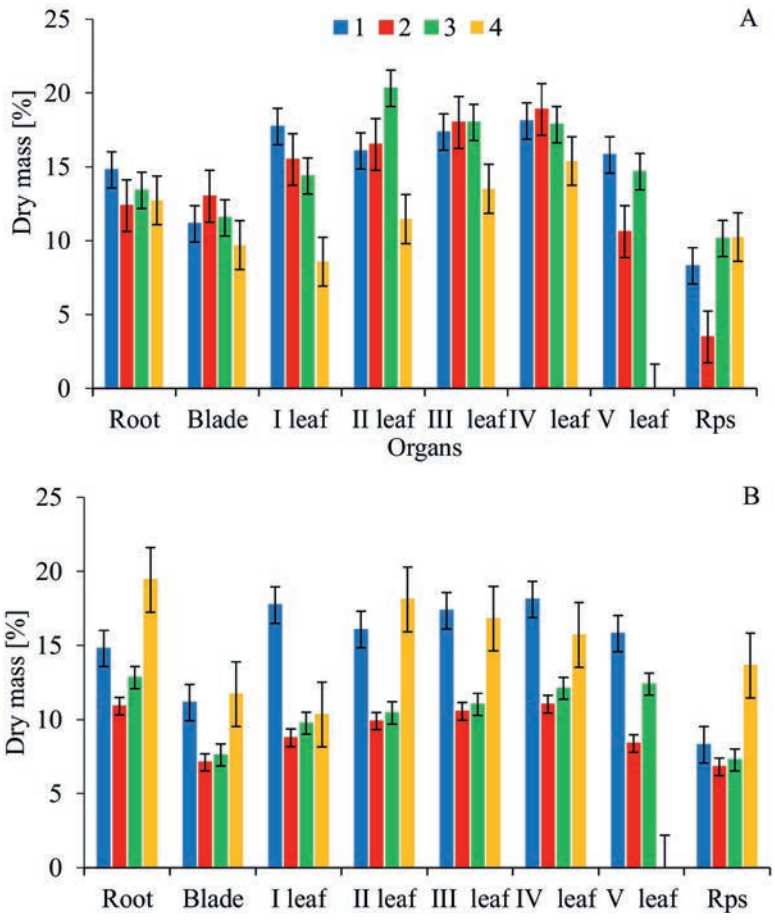

Fig. 2. Development of dry biomass [g] of individual plant organs of *Zea mays* L. in relation to lead application at different concentrations during germination (A) in comparison to treatment after germination during growth (B) (mean values (n = 5); 1 (blue) – control (distilled water), 2 (red) – 0.1% $Pb(NO_3)_2$, 3 (green) – 1% $Pb(NO_3)_2$, 4 (yellow) – 3% $Pb(NO_3)_2$, Rps – Remaining part of the shoot)

depending on time of lead application and concentration of the solution. In most cases, the dry mass of the first maize leaf was higher in plants watered with lead solutions in comparison to the control. For the first leaf no differences were found between the control plants and plants that received 3% $Pb(NO_3)_2$ solution at germination stage or during growth. For the second leaf, the dry mass value was higher in the plants treated with lead solutions compared to the control. The lowest value was observed for plants watered with 3% $Pb(NO_3)_2$ during growth. Dry mass of the third leaf was not affected by lead application during germination while that of the fourth leaf was clearly lower in plants watered with lead solutions and at the highest lead concentration during germination stage. The dry mass of the fifth leaf was significantly lower in each lead treatment what was already obvious in the results presented before. Compared to the control, the dry mass of the remaining part of shoot was lower in all plants watered with lead solutions during growth. During the germination stage, the dry mass of this organ was higher in the treatment with 1% $Pb(NO_3)_2$.

Tab. 4. Development of dry biomass [g] of individual plant organs of *Zea mays* L. in relation to lead application at different concentrations during germination in comparison to treatment after germination (Control – seeds and plants treated with distilled water; lead application: $Pb(NO_3)_2$ water solutions at 0.1%, 1% and 3% concentrations during the germination stage or as irrigation during growth)

Organ	Control	Lead application during germination			Lead application during growth		
		0.1%	1%	3%	0.1%	1%	3%
Root	0.2976 a	0.2618 c	0.2666 c	0.2274 d	0.2290 d	0.2300 d	0.1402 g
Blade	0.0548 b	0.0628 a	0.0464 c	0.0476 c	0.0550 b	0.0643 a	0.0332 d
I Leaf	0.0078 c	0.0110 a	0.0092 b	0.0076 c	0.0106 a	0.0110 a	0.0070 c
II Leaf	0.0244 c	0.0256 bc	0.0312 a	0.0254 bc	0.0302 a	0.0308 a	0.0202 d
III Leaf	0.0523 b	0.0560 b	0.0554 b	0.0500 bc	0.0586 ab	0.0686 a	0.0350 d
IV Leaf	0.0660 ab	0.0700 a	0.0626 ab	0.0300 d	0.0434 c	0.0523 b	0.0174 e
V Leaf	0.0146 a	0.0080 c	0.0088 bc	0 g	0.0028 e	0.0053 d	0 g
Rps	0.0332 b	0.0150 d	0.0438 a	0.0208 c	0.0242 c	0.0300 b	0.0198 d

I Leaf, II Leaf, III Leaf, IV Leaf, V Leaf – leaf number, Rps – Remaining part of the shoot; mean values (n = 5) marked with different letters (within the row) differ significantly according to the Duncan test at $p \leq 0.05$

Water content in the roots and blades of maize was highest in plants watered during growth with lead solutions of 0.1% and 1%, in relation to the remaining concentrations and the control (Tab. 5).

In the case of the blade and the examined leaves, the lead solutions partly caused an increase in the percentage of water content. Statistically significant differences were shown especially in maize plants watered with lead solutions during growth. In the case of the remaining part of the shoot, there were no differences in the water content in relation to lead application.

The intensity of photosynthesis of maize leaves changed in relation to the concentration of lead solutions. With an increase in lead concentration, a decrease in the photosynthetic efficiency of plants was observed (Fig. 3).

Tab. 5. Total water content [%] of individual plant organs of *Zea mays* L. in relation to lead application at different concentrations during germination in comparison to treatment after germination (Control – seeds and plants treated with distilled water; Lead application: $Pb(NO_3)_2$ water solutions at 0.1%, 1% and 3% concentrations during the germination stage or as irrigation during growth)

Organ	Control	Lead application during germination			Lead application during growth		
		0.1%	1%	3%	0.1%	1%	3%
Root	85.21 bc	87.62 b	86.59 bc	87.27 b	89.10 a	87.17 a	80.58 c
Blade	88.85 c	87.00 c	88.46 bc	90.30 b	92.89 a	92.39 a	88.29 b
I Leaf	82.27 b	84.51 b	85.63 b	91.42 a	91.24 a	90.25 a	63.16 c
II Leaf	83.93 b	83.48 b	79.69 bc	88.54 a	90.11 a	89.55 a	81.90 a
III Leaf	82.65 ab	82.00 ab	82.00 ab	86.49 a	89.45 a	88.97 a	83.19 ab
IV Leaf	81.90 b	81.11 b	82.14 ab	84.62 ab	88.97 a	87.89 a	84.30 a
V Leaf	84.20 c	89.39 b	85.33 c	n.d. d	91.62 a	87.62 bc	n.d. d
Rps	91.71 a	96.51 a	89.86 ab	89.75 ab	93.20 a	92.72 a	86.36 ab

I Leaf, II Leaf, III Leaf, IV Leaf, V Leaf – leaf number, Rps – Remaining part of the shoot, n.d. – not developed; mean values (n = 5) marked with different letters (within the row) differ significantly according to the Duncan test at $p \leq 0.05$

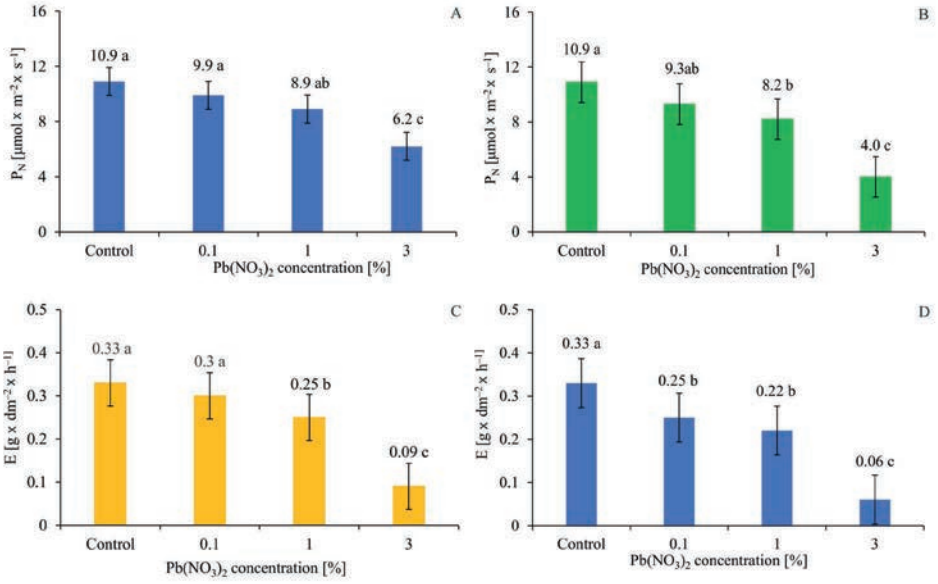

Fig. 3. The intensity of photosynthesis (P_N) and transpiration (E) of the third leaf of *Zea mays* L. grown from grains treated with lead solutions during germination stage and distilled water during growth (A, C), and distilled water during germination and lead solutions during growth (B, D); $Pb(NO_3)_2$ solutions at concentrations 0.1%, 1% and 3%; mean values (n = 5, ± SD) marked with different letters differ significantly according to the Duncan test, with $p \leq 0.05$

In plants grown from grains treated with $Pb(NO_3)_2$ solutions during germination, significant differences were found between 3% solutions and the control sample (Fig. 3A). In plants grown from grains treated with distilled water, and with lead solutions during growth, a negative effect of lead was demonstrated at concentrations of 1% and 3% (Fig. 3B). The intensity of transpiration of *Z. mays* leaves was significantly lower in each of the treatments with lead solutions at germination as well as during growth (Fig. 3.C–D) with the only exception of plants that received 0.1% $Pb(NO_3)_2$ during germination (Fig. 3C).

Discussion

Contamination of soils with heavy metals causes the accumulation of these metals in plant organs, which reduce crop yield (Saifullah et al., 2015). This is confirmed by scientific reports from various regions of the world (Adekunle et al., 2009; Singh et al., 2010; Bigdeli, Seilsepour, 2010; Zhuang et al., 2009; Kachenko, Singh, 2006; Schreck et al., 2013). One of the first stages of plant contact with heavy metals is seeds sown into contaminated soil. During germination, the seed coat becomes permeable not only to water, but also to other environmental factors. It is the first protective barrier that metals pass to get inside the embryo (Kranner, Colville, 2011; Możdżeń, Rzepka, 2016). This study showed the inhibitory effect of lead compounds on the germination of maize (*Zea mays* L.). The negative effect of lead, in the form of nitrate compounds, increased in proportion to the concentration of their solutions (Tab. 1). This kind of response could be related, i.e. with limited water uptake and transport in the presence of lead compounds (Abraham et al., 2013). It could also result from the interference of lead with the enzymes of proteases and amylases (Sengar et al., 2008). Heavy metals in high concentrations contribute to the destabilisation of metabolic processes and the production of excess compounds, harmful to further growth and development (Brewer, 2010; Możdżeń et al., 2017).

Lead, regardless of the form in which it occurs, not only limits seed germination, but also adversely affects plant growth. It inhibits root growth to a greater extent than growth of aboveground plant parts (Greger, 2004; Liu et al., 2009; Shah et al., 2010; Yang et al., 2010; Seregin, Ivanov, 2011). Lead absorption by the roots takes place through the apoplastic route or through calcium ion-permeable channels. After absorption, lead accumulates primarily in the root cells due to the blockage of the "Casparian strips" in the endoderm. Additionally, lead can be picked up by the negative charges on the root cell walls. As the concentration of lead in cells increases, there are also a number of changes at their ultrastructural level (Piechalak et al., 2002).

In the studies carried out here, the negative effect of lead solutions on the growth of maize organs was demonstrated (Fig. 1, 4; Tab. 2). The highest negative effect of lead

was found in plants grown from grains germinated on substrates with the analysed solutions, compared to plants treated with lead solutions only during growth. For example, Shah et al. (2010) found that the seedling stage is more susceptible to heavy metal stress than the later vegetative stages.

The positive values of the IP index indicated the percentage of inhibition of organ growth relative to the control (Fig. 1). These differences, with respect to control, were highest for the fifth leaf and the remaining part of the shoot, and not for the roots. The observed stimulating effect of lead on root growth was most likely a secondary effect, caused by damage to the selective function of cell membranes (Jasiewicz, 1996). Lead uptake by the root system is a passive process and is proportional to the presence of soluble forms in the substrate. The factors that significantly increase its phyto-bio-availability are the acidic pH of the soil and high ambient temperature. Atmospheric lead is an important source of contamination of plants, especially aboveground parts and soils. It is estimated that approximately 73–95% of the total lead content in plants comes from this source (Kabata-Pendias, Pendias, 2001).

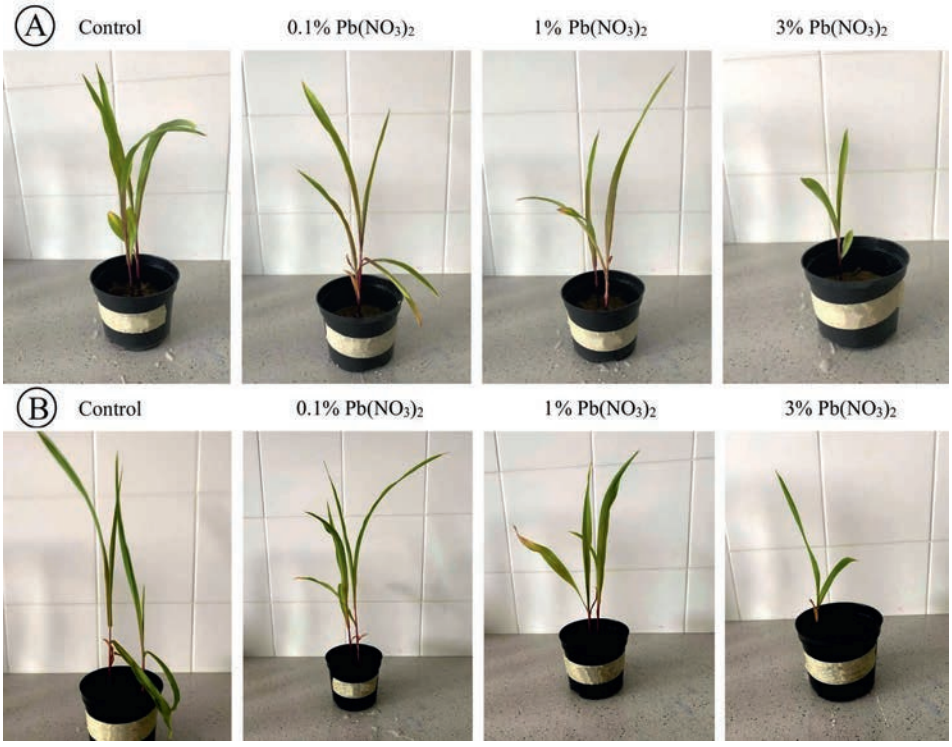

Fig. 4. Maize (*Zea mays* L.) plants grown from grains treated with solutions of $Pb(NO_3)_2$ at concentrations of 0.1%, 1% and 3% at (A) germination and (B) during growth (Photo. E. Bloem)

Ahmad et al. (2011) found the phytotoxic effect of lead on germinating, growth, and the value of fresh and dry mass of maize seedlings. In this study, a decreasing fresh and dry mass and changes in the water content in maize organs was observed (Fig. 2, Tab. 2–5) in relation to lead concentration. The differences in the values of these parameters were proportional to the concentrations of the lead solutions and slightly differed between the chemical compounds from which they were prepared (Ahmad et al., 2011; Malar et al., 2014; Iqbal et al., 2017). The obtained results clearly indicate that maize belongs to plants with poorly developed adaptive mechanisms in relation to high concentrations of lead. The mechanism of tolerance consists primarily in changes in the properties of cell membranes, which, due to the secretion of more pectin's, increase their sorption capacity in relation to lead. Lead can also be excluded from metabolism by retaining it in endoderm cells. One of the processes of lead immobilisation in plants is its precipitation in the form of ortho- and pyrophosphates on the cell membranes of the roots or stems (Jasiewicz, 1996; Sharma, Dubey, 2005).

Lead has a strong influence not only on the morphology but also on the physiology of plants. It causes changes in the structure and functioning of chloroplasts, blocks the electron transport chain, the enzymes of the Calvin cycle, impairs the uptake of essential elements – Mg and Fe, and causes CO_2 deficiency due to the closure of the stomata. Under environmental stress, stomata as well as non-stomata factors can reduce the photosynthetic abilities of plants (Kodera et al., 2008; Oliwa et al., 2016). In the present experiment it was observed that the rate of transpiration (E) influences the photosynthetic ability (Fig. 3). With the increase in the concentration of lead solutions, a decrease in the photosynthetic efficiency of plants was observed. Lower values of parameters were demonstrated in maize plants treated with lead solutions during growth, compared to the control (Fig. 3B). This type of reaction was likely due to excessive evaporation, which can transport the lead ions up the plant and binds them in the surrounding vascular tissue. Perhaps it was also due to the accumulation of lead in the cuticular layer of the leaves (Verbruggen et al., 2009; Puła et al., 2019). The chemical forms of lead have different effects on plants, changing their biological properties. Although they are similarly taken up from the soil, transposed and stored in plant organs, they affect the metabolism to a different extent, in extreme cases leading to death (Iqbal et al., 2017).

Plants have developed strategies to combat heavy metal stress (Hong-Bo et al., 2010). This include, e.g., reduced uptake of metals into the cell, binding of lead to phyto-chelatins, glutathione and amino acids in the vacuole by forming complexes. To achieve nutrient homeostasis, plants have developed various strategies for mobilization and uptake, chelation, storage, and transport between cells and organs. Plants regulate the uptake of nutrients, while responding to changes in the soil and inside (Williams et al., 2000). As a secondary defence system, they have developed antioxidant activation

abilities to combat the increased production of reactive oxygen species (Reddy et al., 2005; Pourrut et al., 2011).

Conclusion

In response to toxic soil conditions, plants struggle to survive by reduced absorption of heavy metals, activating mechanisms that exclude their absorption in metabolically inactive cells. Despite this, they still cannot cope with this type of stress, which is manifested by disturbances in their life processes.

In this experiment, it was observed that lead contamination inhibited the germination of maize grains (*Zea mays* L.) (1), elongation growth of plants organs (2), fresh and dry masses (3) and limited their photosynthetic functions (4). These changes were proportional to the concentration of lead solutions. A negative impact of lead on the morphology and physiology of maize was observed at germination as well as on early growth. At this stage of study, it is difficult to determine at which stage lead was more toxic to plant development.

Conflict of interest

The authors declare no conflict of interest related to this article.

References

Abraham, L.K., Sridevi, R., Suresh, B., Damodharam, T. (2013). Effect of heavy metals (Cd, Pb, Cu) on seed germination of *Arachis hypogeae*. *Asian Journal of Plant Science and Research*, *3(1)*, 10–12.

Adekunle, I.M., Olorundare, O., Nwange, C. (2009). Assessments of lead levels and daily intakes from green leafy vegetables of southwest Nigeria. *Nutrition and Food Science*, *39*, 413–422.

Ahmad, M.S.A., Ashraf, M., Tabassam, Q., Hussain, M., Firdous, H. (2011). Lead (Pb)-induced regulation of growth, photosynthesis, and mineral nutrition in maize (*Zea mays* L.) plants at early growth stages. *Biological Trace Element Research*, *144*, 1229–1239. https://doi.org/10.1007/s12011-011-9099-5

Arnemo, J.M., Andersen, O., Stokke, S., Vernon, G.T., Krone, O., Pain, D.J., Mateo, R. (2016). Health and environmental risks from lead-based ammunition: science versus socio-Politics. *EcoHealth*, *13*, 618–622. https://doi.org/10.1007/s10393-016-1177-x

Bigdeli, M., Seilsepour, M. (2008). Investigation of metals accumulation in some vegetables irrigated with waste water in Shahre Rey-Iran and toxicological implications. *American-Eurasian Journal of Agricultural and Environmental Sciences*, *4(1)*, 86–92.

Brewer, G.J. (2010). Risks of copper and iron toxicity during aging in humans. *Chemical Research in Toxicology*, *23(2)*, 319–326. https://doi.org/10.1021/tx900338d

Carocci, A., Catalano, A., Lauria, G., Sinicropi, M.S., Genchi, G. (2016). Lead toxicity, antioxidant defense and environment. In: F.A. Gunther, P. de Voogt (eds.), Reviews of environmental contamination and toxicology. *Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews)*, vol 238. Switzerland: Springer, Cham. https://doi.org/10.1007/398_2015_5003

Ettler, V. (2016). Soil contamination near non-ferrous metal smelters: A review. *Applied Geochemistry*, *64*, 56–74. https://doi.org/10.1016/j.apgeochem.2015.09.020

Greger, M. (2004). Metal availability, uptake, transport and accumulation in plants. In M.N.V. Prasad (ed.), *Heavy metal stress in plants: from biomolecules to ecosystems*. New York, USA: Springer, pp. 1–21. https://doi.org/10.1007/978-3-662-07743-6_1

Hong-Bo, S., Li-Ye, C., Cheng-Jiang, R., Hua, L., Dong-Gang, G., Wei-Xiang, L. (2010). Understanding molecular mechanisms for improving phytoremediation of heavy metal-contaminated soils. *Critical Reviews in Biotechnology*, *30*(*1*), 23–30. https://doi.org/10.3109/07388550903208057

Hou, D., O'Connor, D., Igalavithana, A.D., Alessi, D.S., Luo, J., Tsang, D.C.W., Sparks, D.L., Yamauchi, Y., Rinklebe, J., Ok., Y.S. (2020). Metal contamination and bioremediation of agricultural soils for food safety and sustainability. *Nature Reviews Earth and Environment*, *1*, 366–381. https://doi.org/10.1038/s43017-020-0061-y

Iqbal, M.M., Murtaza, G., Naz, T., Niazi, N.K., Shakar, M., Wattoo, F.M., Omer Farooq, O., Ali, M., Hafeez-ur-Rehman, Afzal, Mehdi, S.M., Mahmood, A. (2017). Effects of lead salts on growth, chlorophyll contents and tissue concentration of rice genotypes. *International Journal of Agriculture and Biology*, *19*, 69–76.

Jasiewicz, C. (1996). Wpływ ołowiu na plon i skład chemiczny pietruszki (The influence of lead on the yield and chemical composition of parsley). *Zeszyty Problemowe Postępów Nauk Rolniczych*, *434*, 787–792. [In Polish]

Jiao, W., Chen, W., Chang, A.C., Page, A.L. (2012). Environmental risks of trace elements associated with long-term phosphate fertilizers applications: a review. *Environmental Pollution*, *168(1)*, 44–53. http:doi.org/10.1016/j.envpol.2012.03.052

Kabata-Pendias, A., Mukherjee, A.B. (2007). *Trace elements from soil to human*. Berlin-Heidelberg: Springer-Verlag.

Kabata-Pendias, A., Pendias, H. (2001). *Biogeochemistry of trace elements* (*Biogeochemia pierwiastków śladowych*). Warszawa: PWN. [In Polish]

Kachenko, A.G., Singh, B. (2006). Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. *Water Air Soil Pollution*, *169*, 101–123.

Kodera, H., Nishioka, H., Muramatsu, Y., Terada, Y. (2008). Distribution of lead in lead-accumulating pteridophyte *Blechnum niponicum*, measured by synchrotron radiation micro X-ray fluorescence. *Analytical Sciences*, *24(24)*, 1545–1549. http:doi.org/10.2116/analsci.24.1545

Konieczna, I., Rut, G., Kliszcz, A. (2018a). Effect of copper and vanadium ions on morphology of carrot (*Daucus carota* L. subsp. *sativus* (Hoffm.) Schübl. & G. Martens) and wheat (*Triticum aestivum* L.) plants. *Annales Universitatis Paedagogicae Cracoviensis Studia Naturae*, *3*, 55–69. https://doi.org/10.24917/25438832.3.4

Konieczna, I., Rut, G., Kliszcz, A. (2018b). Photosynthetic activity of *Daucus carota* L. subsp. *sativus* (Hoffm.) Schübl. & G. Martens and *Triticum aestivum* L. in the presence of copper and vanadium ions. *Annales Universitatis Paedagogicae Cracoviensis Studia Naturae*, *3*, 70–79. https://doi.org/10.24917/25438832.3.5

Kranner, I., Colville, L. (2011). Metals and seeds: biochemical and molecular implications and their significance for seed germination. *Environmental and Experimental Botany*, *72*, 93–105. https://doi.org/10.1016/j.envexpbot.2010.05.005

Li, X.M., Bu, N., Li Y., Ma, L., Xin, S., Zhang, L. (2012). Growth, photosynthesis and antioxidant responses of endophyte infected and non-infected rice under lead stress conditions. *Journal of Hazardous Materials*, *213–214(3)*, 55–61. http:doi.org/10.1016/j.jhazmat.2012.01.0522

Liu, T., Liu, S., Guan, H., Ma, L., Chen, Z., Gu, H., QU, L.-J. (2009). Transcriptional profiling of *Arabidopsis* seedlings in response to heavy metal lead (Pb). *Environmental and Experimental Botany*, *67(2)*, 377–386. https://doi.org/10.1016/j.envexpbot.2009.03.016

Malar, S., Manikandan, R., Favas, P.J.C., Vikram, S., Sahi, S.E., Venkatachalam, P. (2014). Effect of lead on phytotoxicity, growth, biochemical alterations and its role on genomic template stability in *Sesbania grandiflora*: A potential plant for phytoremediation. *Ecotoxicology and Environmental Safety*, *108*, 249–257. https://doi.org/10.1016/j.ecoenv.2014.05.018

Marcinek, J., Komisarek, J., Kazmierowski, C. (1995). Physical soil degradation of infensive farming hapludalfs and endoaquolls in Great Poland (Wielkopolska) (Degradacja fizyczna gleb płowych i czarnych ziem intensywnie użytkowanych rolniczo w Wielkopolsce). *Zeszyty Problemowe Postępów Nauk Rolniczych*, *418(1)*, 141–147. [In Polish]

Mominul Islam, A.K.M., Kato-Noguchi, H. (2012). Allelopathic potentiality of medicinal plant *Leucas aspera*. *International Journal of Agricultural Sustainability*, *4*, 1–7.

Możdżeń, K., Barabasz-Krasny, B., Puła, J., Lepiarczyk, A. (2017). Influence of cadmium and zinc compounds on the early stages of radish (*Raphanus sativus* L. var. *sativus*) growth (Wpływ związków kadmu i cynku na wczesne etapy wzrostu rzodkiewki (*Raphanus sativus* L. var. *sativus*)). *Fragmenta Agronomica*, *34(2)*, 45–54. [In Polish]

Możdżeń, K., Rzepka, A. (2016). The role of seed coat during germination and growth of broad bean (*Vicia faba* L.) seeds in the presence of lead sulphate (Rola łupiny nasiennej podczas kiełkowania i wzrostu nasion bobu (*Vicia faba* L.) w obecności siarczanu ołowiu. *Annales Universitatis Mariae Curie-Skłodowska. Sectio E, Agricultura*, *71*, 55–65. [In Polish]

Możdżeń, K., Wanic, T., Rut, G., Łaciak, T., Rzepka, A. (2017). Toxic effects of high copper content on physiological processes in *Pinus sylvestris* L. *Photosynthetica*, *55(1)*, 193–200. https://doi.org/10.1007/s11099-016-0229-3

Oliwa, J., Możdżeń K., Migdałek, G., Wanic, I., Bojarski, B., Rut, G., Rzepka, A. (2016). Physiological activity of cucumber (*Cucumis sativus* L.) in cadmium stress. In M.T. Grzesiak, A. Rzepka, T. Hura, S. Grzesiak (ed.), *Plant functioning under environmental stress*. Cracow: The F. Górski Institute of Plant Physiology: Polish Academy of Sciences, s. 95–104.

Pattee, O.H., Pain, D.J. (2002). Lead in the environment. In D.J. Hoffman, B.A. Rattner, G.A. Burton Jr., J. Cairns Jr. (eds.). *Handbook of ecotoxicology*. Boca Raton: CRC Press, pp.36.

Piechalak, A., Tomaszewska, B., Baralkiewicz, D., Malecka, A. (2002). Accumulation and detoxification of lead ions in legumes. *Phytochemistry*, *60*, 153–162. http://doi.org./0.1016/S0031-9422(02)00067-5

Pourrut, B., Shahid, M., Dumat, C., Winterton, P., Pinelli, E. (2011). Lead uptake, toxicity, and detoxification in plants. *Reviews of Environmental Contamination and Toxicology*, *213*, 113–136. http://doi.org/10.1007/978-1-4419-9860-6_4

Puła, J., Barabasz-Krasny, B., Lepiarczyk, A., Zandi, P., Możdżeń, K. (2019). Activity of the photosynthetic apparatus in *Phaseolus vulgaris* L. leaves under the cadmium stress. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *47(2)*, 405–411. http://doi.org/10.15835/nbha47111328

Reddy, A.M., Kumar, A.G., Jyothsnakumari, G., Thimmanaik, S., Sudhakar, C (2005). Lead induced changes in antioxidant metabolism of Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) and Bengal gram (*Cicer arietinum* L.). *Chemosphere*, *60(1)*, 97–104. https://doi.org/10.1016/j.chemosphere.2004.11.092

Rodriguesa, A.A.Z., De Queiroz, M.E.L.R., Oliveira, A.F., Heleno, A.A.F.F., Zambolim, L., Freitasa, J.F., Morais, E.H.C. (2017). Pesticide residue removal in classic domestic processing of tomato and its effects on product quality. *Journal of Environmental Science and Health. Part. B*, *52*, 1–8. https://doi.org/10.1080/03601234.2017.1359049

Saifullah, S.M., Zia-Ur-Rehman, M., Sabir, M., Ahmad, H.R. (2015). Phytoremediation of pb-contaminated soils using synthetic chelates. In K. Hakeem, M. Sabir, M. Ozturk, A. Murmet (eds.), *Soil remediation and plants*. San Diego: Elsevier, pp. 397–414. https://doi.org/10.1016/B978-0-12-799937-1.00014-0

Schreck, E., Laplanche, C., Le Guédard, M., Bessoule, J.-J., Austruy, A., Xiong, T., Foucault, Y., Dumat, C. (2013). Influence of fine process particles enriched with metals and metalloids on *Lactuca sativa*

L. leaf fatty acid composition following air and/or soil-plant field exposure. *Environmental Pollution*, *179*, 242–249. https://doi.org/10.1016/j.envpol.2013.04.024

Sengar, R.S., Gautam, M., Sengar, R.S., Garg, S.K., Sengar, K., Chaudhary, R. (2008). Lead stress effects on physiobiochemical activities of higher plants. *Reviews of Environmental Contamination and Toxicology, 196*, 73–93. http:/doi.org./10.1007/978-0-387-78444-1_3

Seregin, I.V., Ivanov, V.B. (2001). Physiological aspects of cadmium and lead toxic effects on higher plants. *Russian Journal of Plant Physiology*, *48(4)*, 523–544

Shah, F.R., Ahmad, N., Masood, K.R., Peralta-Videa, J.R., Ahmad, F.D. (2010). Heavy metal toxicity in plants. In M. Ashraf, M. Ozturk, M.S.A. Ahmad (eds.), *Plant adaptation and phytoremediation*. New York: Springer, pp. 71–97.

Sharma, P., Dubey, R.S. (2005). Lead toxicity in plants. *Brazilian Journal of Plant Physiology*, *17(1)*. https://doi.org/10.1590/S1677-04202005000100004

Singh, R., Tripathi, R.D., Dwivedi, S., Kumar, A., Trivedi, P.K., Chakrabarty, D. (2010). Lead bioaccumulation potential of an aquatic macrophyte *Najas indica* are related to antioxidant system. *Bioresource Technology*, *101*, 3025–3032. https://doi.org/10.1016/j.biortech.2009.12.031

Steiner, A.A. (1961). A universal method for preparing nutrient solutions of a certain desired composition. *Plant and Soil*, *15(2)*, 134–154.

Verbruggen, N., Hermans, C., Schat, H. (2009). Molecular mechanisms of metal hyperaccumulation in plants. *New Phytologist*, *181(4)*, 759–776. http://doi.org/10.1111/j.1469-8137.2008.02748.x

Williams, L.E., Pittman, K.J., Hall, J. (2000). Emerging mechanisms for heavy metal transport in plants. *Biochimica et Biophysica Acta-Biomembranes*, *1465*, 104–126. http://doi.org/10.1016/S0005-2736(00)00133-4

Yang, Y., Wei, X., Lu, J., You, J., Wang, W., Shi, R. (2010). Lead-induced phytotoxicity mechanism involved in seed germination and seedling growth of wheat (*Triticum aestivum* L.). *Ecotoxicology and Environmental Safety*, *73*, 1982–1987. https://doi.org/10.1016/j.ecoenv.2010.08.041

Zhou, J., Zhang, Z., Zhang, Y., Wei, Y., Jiang, Z. (2018). Effects of lead stress on the growth, physiology, and cellular structure of privet seedlings. *PLoS One*, *13(3)*, e0191139. https://dx.doi.org/10.1371%2Fjournal.pone.0191139

Zhuang, P., McBride, M.B., Xia, H., Li, N, Li, Z. (2009). Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine. *South China Science Total Environment*, *407*, 1551–1561. https://doi.org/10.1016/j.scitotenv.2008.10.061

Abstract

Soil contamination with heavy metals leads to the accumulation of significant amounts of these elements in plants and disrupts their growth and development. The current experiment investigated the effect of lead in the form of $Pb(NO_3)_2$ in water solutions of various percentages (0.1%, 1%, 3%) on the germination of maize grains (*Zea mays* L.), plant growth (fresh and dry mass) and their photosynthetic activity. The experiment was performed on plants grown from grains germinated on lead solutions and on plants germinated in distilled water, and watered with lead solutions during growth. The negative influence of lead solutions on the germination capacity of grains was demonstrated. Regardless of the timing of lead application, maize elongation growth was clearly inhibited. Similar results were obtained for the masses of the examined plant organs. The rate of transpiration influenced the photosynthesis intensity and depended on the concentration of the lead solution. Along with the increase in lead concentrations a negative effect of lead on all the parameters tested was observed. In general, it can be concluded that only proper management of arable soils can limit the uptake of heavy metals by plants and thus improve their growth and development.

Key words: elongation growth, germination, masses, photosynthesis, transpiration

Received: [2021.08.01]
Accepted: [2021.10.06]

Wpływ związków ołowiu na wybrane cechy morfologiczne i procesy fizjologiczne *Zea mays* L.

Streszczenie

Skażenie gleb metalami ciężkimi prowadzi do kumulowania znacznych ilości tych pierwiastków w roślinach oraz zaburzeń ich wzrostu i rozwoju. W doświadczeniu zbadano wpływ ołowiu, w postaci wodnych roztworów $Pb(NO_3)_2$ o różnych stężeniach procentowych (0.1%, 1%, 3%), na kiełkowanie ziarniaków kukurydzy (*Zea mays* L.), wzrost roślin (świeżą i suchą masę) oraz ich aktywność fotosyntetyczną. Eksperyment wykonano na roślinach wykiełkowanych na roztworach z ołowiem i na roślinach wykiełkowanych na wodzie destylowanej, a podlewanych roztworami ołowiu w czasie wzrostu. Wykazano negatywny wpływ roztworów ołowiu na zdolność kiełkowania ziarniaków. Niezależnie od etapu podlewania, wzrost elongacyjny kukurydzy był wyraźnie hamowany. Podobne rezultaty uzyskano dla mas badanych organów roślin. Szybkość transpiracji wpływała na intensywność fotosyntezy i zależała od stężenia roztworu ołowiu. Wraz ze wzrostem stężeń ołowiu, obserwowano jego negatywny wpływ na wszystkie badane parametry. Generalnie można stwierdzić, że tylko właściwe gospodarowanie glebami uprawnymi może ograniczyć pobieranie metali ciężkich przez rośliny i tym samym poprawić ich wzrost oraz rozwój.

Słowa kluczowe: kiełkowanie, wzrost elongacyjny, masa, fotosynteza, transpiracja

Information on the authors

Elke Bloem https://orcid.org/0000-0002-5597-1969
Her research focuses on the metabolism of sulfur in plants and soils. The investigation of secondary sulfur--containing compounds and valuable plant ingredients in relation to biotic (fungal pathogens) and abiotic stress (salt and drought) are topics of collaboration. It is the main target to understand which factors (biotic and abiotic) are important in secondary plant metabolism and how this knowledge can be transferred to agriculture to deliver high-value medical crops and healthy plants.

Jowita Ciaranek
She was am MSc degree student of biology of the Pedagogical University in Kraków (Poland). She is interested in the impact of heavy metals on plant physiology.w