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INFLUENCE OF LEAD ACCUMULATION ON PHYSIOLOGICAL PARAMETERS AND MINERAL ELEMENT (Mg, Fe, Mn) UPTAKE IN CROP PLANTS

Vita Alle[1], Anita Osvalde[2], Uldis Kondratovics[1], Mara Vikmane[1]
[1] *University of Latvia, Faculty of Biology,*
[2] *University of Latvia, Institute of Biology, Laboratory of Plant Mineral Nutrition*

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Abstract

Soil pollution with hazardous elements including heavy metals has become a problem all around the world. Heavy metals can cause health problems for people through access to the food chain. So, it is important to pay attention to soil, especially agricultural soil, contamination with hazardous elements. Mechanisms of physiological processes in plants are affected under heavy metal pollution. The aim of this study was to find out the impact of different lead (Pb) concentrations in the substrate on Pb accumulation in barley and lettuce leaves and roots, uptake of mineral elements, as well as effect on photosynthesis and chlorophyll *a* fluorescence, thus revealing differences between monocotyledons and dicotyledons. Barley (*Hordeum vulgare* L.) and lettuce (*Lactuca sativa* L.) were selected for the vegetation experiment as representatives of the monocotyledons and dicotyledons, respectively. Plants were grown up in quartz sand under controlled growth conditions. The experiment lasted 28 days for barley and 43 days for lettuce. Plant growth and physiological parameters were investigated under increasing level of Pb in substrate: 0, 400, 600, 800, 1000 mg L^{-1} for barley and 0, 200, 250, 300, 350 mg L^{-1} for lettuce. Pb was added as $Pb(NO_3)_2$ in substrate. The following methods were used to analyze the plant material: the concentrations of Pb, Mg, Fe and Mn in air-dry plant material were estimated by atomic absorption spectrophotometry (Perkin Elmer AAnalyst 700); the content of photosynthetic pigments were determined by spectrophotometry method; chlorophyll *a* fluorescence parameters were determined with continuous excitation chlorophyll fluorimeter Handy PEA system. It was observed that the fresh weight of the experimental plants decreased with increasing concentration of lead in the substrate. Pb concentrations in roots were higher than in leaves for both barley and lettuce. There were differences in the ability of Pb accumulation between model object leaves during the experiment. The results showed that in the conditions of the highest Pb concentrations in the substrate respectively 1000 mg L^{-1} for barley, the concentration of Pb in barley leaves was 414.20 mg kg^{-1}, while three timed lower pollution level for lettuce (Pb 350 mg L^{-1}), caused almost similar Pb in lettuce leaves - 329.74 mg kg^{-1}. In general, the uptake of several mineral elements (Mg, Fe, Mn) in lettuce and barley increased under Pb pollution. An increase of Pb concentrations in substrate resulted in the decreased content of chlorophyll *a+b* in leaves of model objects. At the end of the experiment in the conditions of maximum investigated Pb concentrations the content of chlorophyll *a+b* in barley leaves was 2.1 times lower and in lettuce leaves 1.3 times lower than that in the control plant leaves. The results showed that increasing Pb concentrations in substrate has a different effect on photosynthesis describing parameters in monocotyledons and dicotyledons. Both exclusion and tolerance strategies operate as plant resistance mechanisms to Pb as a stress factor in the model objects – barley and lettuce.
Keywords: dicotyledon, heavy metals, monocotyledon, photosynthesis parameters

Introduction

Soil pollution with hazardous elements including heavy metals has become a problem all around the world (He et al, 2005; Sękara et al, 2005; Pourrut et al, 2011). There are two ways for soil pollution with heavy metals - as a natural process or as an anthropogenic activity (Stančić et al, 2016). For example, soil contamination with heavy metal in natural processes - storm-induced soil and mountain rock erosion, volcanic activity, evaporation from the seas and oceans, forest fires, cosmic dust, biological processes and anthropogenic activity - mine mining, metallurgy, energetics, chemical industry, transport, agriculture (He et al, 2005; Titov et al, 2007; Peralta-Videa et al, 2009; Yadav, 2010; Meuser and Van de Graaff, 2011; Swartjes, 2011). According to Van Liedekerke et al. (2014) in Europe the most frequently occurring contaminant groups in soil are heavy metals (35%), mineral oils (24%), polycyclic aromatic hydrocarbons (11%), aromatic hydrocarbons (10%), chlorinated hydrocarbons (8%), phenols (1%), cyanides (1%), others (10%). According to data from the European Environmental Agency there are 2.5 million potentially contaminated sites in Europe (van Liedekerke et al, 2014). This indicates the importance of soil pollution as problem in Europe. A study by Burlakovs and Vircavs (2012) revealed that there are 56 sites contaminated with heavy metals also in Latvia. As heavy metals can cause health problems for people through access to the food chain (Peralta-Videa et al, 2009; Tóth et al, 2016), it is important to pay attention to soil, especially agricultural soil, contamination with hazardous elements.

Among heavy metals, Pb is one of the most toxic and common in environment, because it is widely used in industrial processes (Pourrut et al, 2011) and is nondegradable. Pb has low water solubility and availability for plant uptake (Peralta-Videa et al, 2009), uptake of Pb ions by roots is a passive process (Musielińska et al, 2016). Pb can damage such body systems as central nervous and cardiovascular system in mammals (Peralta-Videa et al, 2009).

According to literature heavy metals can cause inhibition of plant metabolism, reduce uptake of nutrients, have adverse impact on growth rate, reproduction, and root vitality (Cheng, 2003), reduce division and elongation of root cells (Kozhevnikova et al, 2009). At high concentrations Pb reduce plant growth, development and productivity (He et al, 2005), as well as alter uptake of essential nutrients, so the translocation of nutrients in plants is affected by plant roots (endodermis layer) (Barberon, 2017). Several studies have reported that toxic level of Pb increase Mg level (Siedlicka, 1995) but decrease Fe, K, Ca level in plant roots or shoots (Siedlicka, 1995; Kaur et al, 2013), because Pb blocks the entry of cations into roots (Kaur et al, 2013). The process of photosynthesis is involved by mineral elements, for example, magnesium, iron, and manganese (Sengar et al, 2008; Pourrut et al, 2011), and it is therefore important to look at the amount of these elements in plant leaves in relation to the influence of heavy metal Pb on the photosynthesis process.

In general, heavy metal impact on plants is species-specific and depends on their concentration and bioavailability in the soil, environmental conditions, plant population (Musielińska et al, 2016; Stančić et al, 2016). The growth of whole plants or plant parts are frequently used as an easily measurable parameters to monitor the effects of various stressors including heavy metals. Changes in growth are often the first and most obvious reactions of plants under stress. The organs that have the first direct contact with harmful substances, generally the roots in the contaminated soils, show rapid changes in their growth characteristics. In plants visual symptoms caused by heavy metals are chlorosis of leaf and root necrosis, colour darkening of roots, reduction of root diameter and branching (Gil et al, 1995; Pourrut et al, 2011; Capelo et al, 2012, Singh et al, 2015; Jyothsna and Murthy, 2016).

Heavy metals can decrease root respiration, active transport of elements, cause root damage, hinder root growth (decrease unsuberinized root tips area, the permeability barrier of root cells are destroyed) (Siedlecka, 1995). It should be noted that interaction mechanisms of mineral elements could be different under heavy metal conditions (Siedlecka, 1995; Musielińska et al, 2016).

Chirkova (2002) has described two resistance mechanisms of plants to heavy metals:

1. preventing the penetration of ions into the cell - immobilization of ions in the cell wall; inhibition of transport across the plasmalemma; secretion of metal chelating ligands;
2. intracellular mechanisms of tolerance to heavy metals:
   - 2.1. detoxification – complex synthesis with organic acids, metallothioneins, phytochelatins, ferritins, glutathione; heavy metal excretion into vacuoles;
   - 2.2. metabolism reparation;
   - 2.3. functioning in the presence of heavy metals – alternative metabolism; synthesis of heavy metal tolerant enzymes.

There are few information in literature about Pb uptake mechanisms into plant. Wierzbicka (1999) has reported that *Hordeum vulgare*, *Zea mays* and *Allium cepa* tolerate Pb through complexation and inactivation, and has found that Pb is bound to carboxylic groups of mucilage uronic acids on root surfaces and that lead is mainly accumulated in the roots of plants. Seregin and Ivanov (2001) have reported that the major barriers are the endodermis and the cell walls of the central cylinder that restrain the uptake of heavy metals into the stelar cells. Also Pourrut et al. (2011) have found that a limited amount of lead is moved from roots to leaves, this is provided by the root endodermis – which executes the barrier function (for example, Casparian strips). Capelo et al. (2012) have wrote that endodermis has a negative effect on Pb transport mechanisms from roots to shoots. Líška et al. (2016) describe negative impact of root endodermis on heavy metal translocation from roots to shoots. This could be the reason for differences in the amount of Pb in the leaves between the monocotyledons and dicotyledons. According to the literature (Sękara et al, 2005), the roots of monocotyledons accumulate more lead (mg kg^{-1} dry weight) than shoots (roots – barley 13.14, maize 8.12; shoots - barley 2.81, maize 1.75) but the shoots of dicotyledons accumulate more lead than roots (roots - red beet 2.44, field pumpkin 4.49, alfalfa 8.44; shoots – red beet 8.71, field pumpkin 7.55, alfalfa 11.33).

There are evidences that Pb has more negative impact on corns, beans, lettuce and radishes growing on calcareous soils (Sengar at al, 2008), so Pb uptake in plants also depends on soil pH value. Negative effect of Pb on plants is shown up as reduction of seedling growth (barley, maize, rice, tomato, legumes), root and stem elongation, leaf expansion, for example, for barley, *Allium* species, *Raphanus sativus* (Sengar at al, 2008; Pourrut et al, 2011), so Pb cause reduction of plant growth (Pourrut et al, 2011; Capelo et al, 2012; Musielińska et al, 2016).

According to the literature, Pb toxicity resulted in photosynthesis inhibition (Sengar et al, 2008; Nagajyoti et al, 2010; Pourrut et al, 2011; Leal-Alvarado et al, 2016). Changed effect of the stomata, destroyed structure of chloroplasts – similarity of Pb for protein N and S ligands (Leal-Alvarado et al, 2016), biochemical changes in the metabolic products of photosynthesis resulted into reduction of photosynthesis. Pb has inhibitory effect on carboxylating enzymes, but has stimulatory effect on chlorophyllase activity. Pb can modify lipid composition of thylakoid membranes, can reduce activities of Calvin cycle enzymes, reduce electron transport, reduce synthesis of chlorophyll, carotenoids, plastoquinone, activity of $NADP^+$ oxidoreductase (Sengar at al, 2008; Leal-Alvarado et al, 2016). The inhibitory effect of lead is less appeared in photosystem I (PS I) electron transport than in photosystem II (PS II). Previous studies showed that Pb decrease chlorophyll content in oats, cucumber and maize (Sengar at al, 2008).

Chlorophyll *a* fluorescence parameters are widely used to investigate photosynthesis of plants. Due to its sensitivity to changes of photosynthesis (Kalaji et al, 2014), chlorophyll *a* fluorescence parameters can be used to determine the effects of the environment on the photosynthesis process (Sayed, 2003). This is non-destructive method to investigate impact of several stressors on photosynthesis apparatus (Appenroth et al, 2011; Kalaji et al, 2014). The variable fluorescence F_v/F_m represents maximum quantum yield of PS II photochemistry in dark-adapted leaves and Performance Index (P_{Index}) represents the vitality of the plant (Kalaji et al, 2014).

The aim of this study was to find out the impact of different lead (Pb) concentrations in the substrate on Pb accumulation in barley and lettuce leaves and roots, uptake of mineral elements, as well as the effect on photosynthesis and chlorophyll *a* fluorescence, thus revealing differences between monocotyledons and dicotyledons.

Materials and methods

Barley (*Hordeum vulgare* L., cv. 'Ansis') and lettuce (*Lactuca sativa* L., cv. 'Grand Rapid') were selected for the vegetation experiment as representatives of the monocotyledons and dicotyledons, respectively. Plants were grown up in quartz sand filled in 1 L polyethylene containers from seeds (18 barley plants per container and three lettuce plants per container) under controlled growth conditions: day/night temperature + 20/18 °C, photoperiod light/dark 16/8 h, moisture of substrate 60-65%, a photon flux density of 160 µmol m^{-2} s^{-1} supplied by fluorescent tubes. Moisture of the substrate was maintained over the experiment using deionized water.

The vegetation experiment lasted 28 days for barley and 43 days for lettuce. Plant growth and physiological parameters were investigated under increasing level of Pb in substrate: 0, 400, 600, 800, 1000 mg L^{-1} for barley and 0, 200, 250, 300, 350 mg L^{-1} for lettuce. Pb was added as $Pb(NO_3)_2$ in substrate. Nutrient solution, containing optimal concentrations of macronutrients and micronutrients (in mg L^{-1}: N 120, P 60, K 150, Ca 800, Mg 50, S 60, Mn 1.5, Zn 1, Cu 0.5, Mo 0.02, B 0.2, Fe 30) (Osvalde, 2011), was added in substrate for control treatment group. All the nutrients were provided with complete nutrient solution. As the lead standard solution contains nitrogen, adjustments were done to prepare the complete nutrient solutions for Pb treatments by reducing the content of ammonium nitrate. Ca was added as $CaCO_3$ two grams in substrate of each container at the beginning of the experiment. The variants of the experiment were arranged a completely randomized. Designations of experiment variants: Pb 0 (control treatment, lead was not added in the substrate), Pb 400 (lead 400 mg L^{-1} was added in substrate), Pb 600 (lead 600 mg L^{-1} was added in substrate) *et cetera*.

Plants were collected on the day of 8th, 14th, 18th, 22nd and 28th in the experiment of barley and on the day of 25th, 29th, 33rd, 37th and 43rd in the experiment of lettuce. Biomass of plants was determined throughout the experiment. Roots of plants were separated from shoots and washed in distilled water. For each plant fresh weight of leaves and roots was determined.

Content of photosynthetic pigment – amount of chlorophyll *a+b* – in model object leaves was determined by spectrophotometry method using JENWAY 6300 Spectrophotometer (JENWAY, UK). Amount of chlorophyll *a+b* was determined in the first fully expanded leaves of plants. Segment of a fresh leave was crushed in a pestle, pigments were extracted with 20 mL of 96% ethanol. Extracts were centrifuged at 5000 rpm for 5 minutes and absorbances were measured at 470, 649 and 664 nm. Finally amount of chlorophyll *a+b* was calculated after Lichtenthaler (1987) equitation.

Chlorophyll *a* fluorescence parameters were determined with continuous excitation chlorophyll fluorimeter Handy PEA system (Hansatech, UK). Leaf clips were put on leaves for dark-adaptation and it lasted 20 min. The following chlorophyll *a* fluorescence parameters were determined: the variable fluorescence (F_v/F_m) and Performance Index (P_{Index}).

Plant material was dried at 64°C to a constant weight. The concentrations of lead (Pb), magnesium (Mg), iron (Fe) and manganese (Mn) in air-dry plant (DW) material were estimated by an atomic absorption spectrometer Perkin Elmer AAnalyst 700A, acetylene-air flame. Plant material was dry mineralized with HNO_3 vapours and dissolved in HCl (Rinkis et al, 1987). For control treatment Pb analyses were conducted on a graphite furnace equipped atomic absorption spectrometer Perkin Elmer AAnalyst 700 (Anonymous, 2000).

The statistical analysis of results was done using MS Excel 2013. Standard errors (SE) were calculated in order to reflect the mean of the results. All chemical analyzes were done in three replicates.

Results

Barley and lettuce exposure to increasing Pb levels in substrate resulted in decreasing fresh biomass of plant leaves and roots. There were different pattern of plant biomass reduction for two model plant species studied.

At the last experiment day biomass of barley leaves was 42.24% and of roots 91.30% of the control (Pb 0) at the highest pollution level (Pb 1000 mg L^{-1}) (Figure 1, A, B). The fresh weight of barley leaves for all Pb treatments was lower than that of the control variant throughout the experiment. In contrast, the fresh weight of barley roots on the 8th day of the experiment was higher than that of the control variant for all treatments. Thus, the fresh weight of barley roots for the treatment Pb 1000 mg L^{-1} reached 158.68% of the control level (Figure 1, B). Until the end of the experiment, the fresh weight of the roots decreased under the control level for all treatments and was 97.83% (Pb 400), 93.48% (Pb 600), 96.74% (Pb 800) and 91.30% (Pb 1000) of the control (Pb 0) (Figure 1, B). Among the variants of the experiment there were significant differences in the fresh weight of the barley

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leaves throughout the experiment ($p<0.05$). At the end of the experiment (the 22nd and the 28th day), there was no significant differences in the fresh weight of the barley roots among the variants of the experiment.

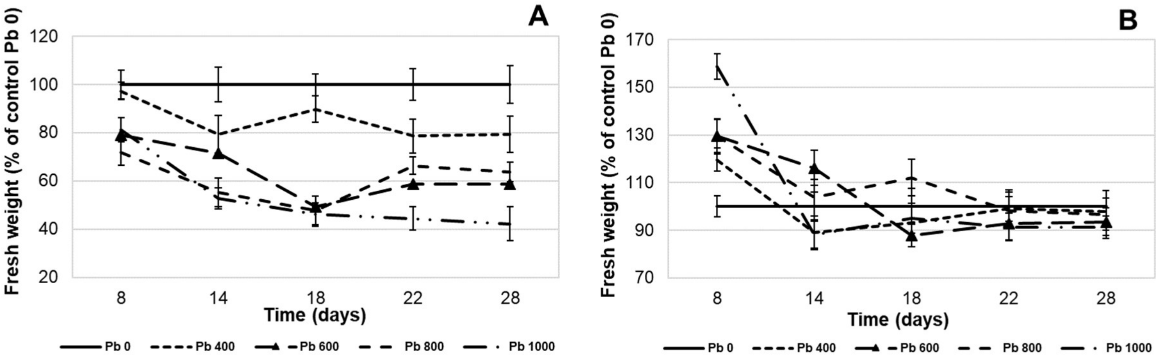

Figure 1. Fresh weight (% of control Pb 0) of the leaves (A) and roots (B) of *H. vulgare* L. at five levels of Pb added in substrate, ±SE

Unlike to barley, the fresh weight of lettuce leaves and roots decreased similarly throughout the experiment (Figure 2, A, B). The treatment of Pb 350 mg L^{-1} resulted in the inhibition of leaf and root biomass till 3.43% and 6.19%, respectively, of the control level at the end of the experiment (the 43rd day). It is notable that at higher pollution levels of substrate (Pb 300 and Pb 350) a significant fall in the fresh weight of lettuce leaves and roots occurred if compared to lower pollution levels (Pb 200 and Pb 250) throughout the experiment ($p<0.05$) (Figure 2, A, B).

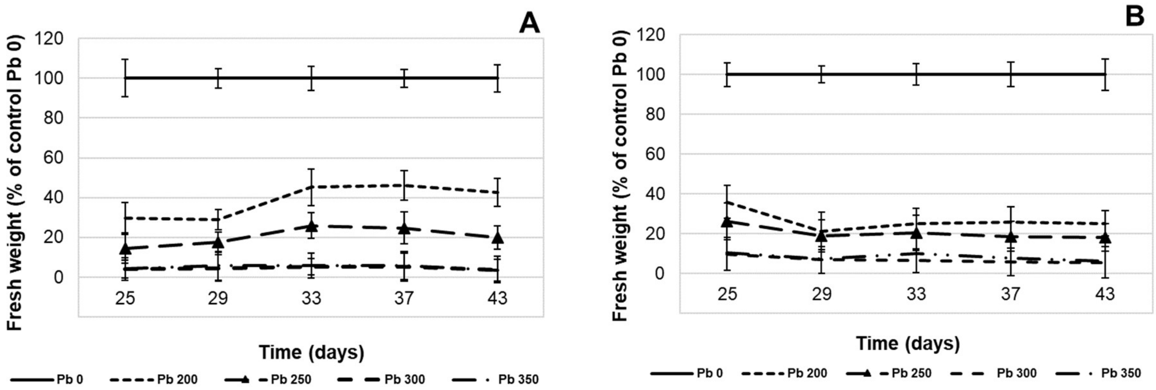

Figure 2. Fresh weight (% of control Pb 0) of the leaves (A) and roots (B) of *L. sativa* L. at five levels of Pb added in substrate, ±SE

Plant supply with increasing levels of Pb caused impact not only on plant biomass but also on plant development. At the end of the experiment, barley had grown up to the 6th leaf stage (at control condition Pb 0), while plants exposed to all Pb treatments (Pb 400, Pb 600, Pb 800, Pb 1000) reached the 4th leaf stage. Different situation was observed in the experiment with model object lettuce - at control condition (Pb 0) lettuce had grown up to the 9th leaf stage, while at Pb 200 - to the 6th leaf stage, at Pb 250 - to the 5th leaf stage, and finally at Pb 300 and Pb 350 - to the 4th leaf stage.

The impact of increased Pb levels on the content of chlorophyll *a+b* in plant leaves was different for both species studied. For barley, throughout the vegetation experiment, the chlorophyll *a+b* content in the control plant leaves was higher than that of the all Pb treatments (Figure 3, A). At the end of the experiment, under the highest level of Pb in substrate (1000 mg L^{-1}), the content of chlorophyll *a+b* in barley leaves was 1.10 mg g^{-1} and in the control plants 2.29 mg g^{-1} (the content of chlorophyll *a+b* in barley leaves was 52% lower than that in the control plants) (Figure 3, A).

Plant exposure to Pb pollution level 200 and 250 mg L^{-1} in the substrate resulted in the small increase 0.62 – 0.70 mg g^{-1} (6%-17%) in the chlorophyll *a+b* content in lettuce leaves on the 33rd and 37th day of the experiment (Figure 3, B). At the beginning and the end of the experiment this impact was not established. The highest

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level of Pb in substrate (350 mg L^{-1}) reduced the content of chlorophyll *a+b* in lettuce leaves for 0.12 mg g^{-1} (for 23%) at the end of the experiment (Figure 3, B).

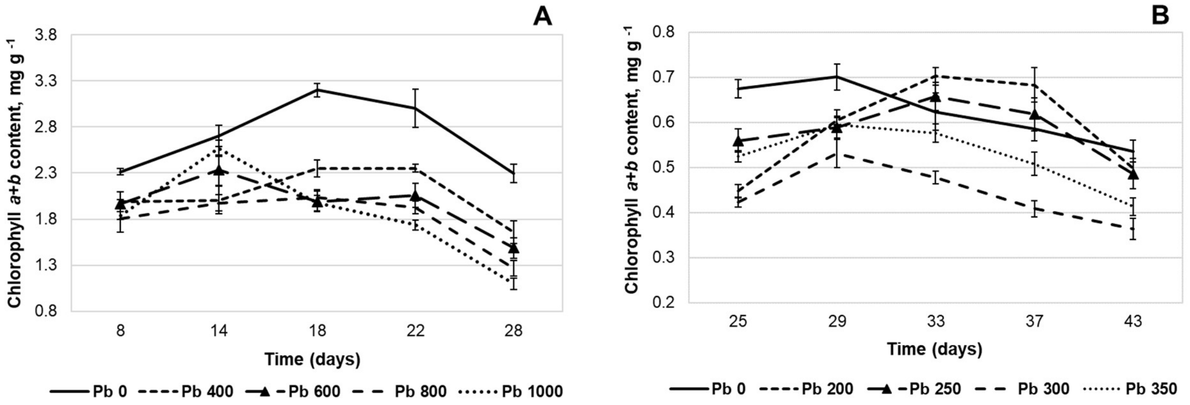

Figure 3. Chlorophyll *a+b* content (mg g^{-1}) in *H. vulgare* L. (A) and *L. sativa* L. (B) leaves at five levels of Pb added in substrate, ±SE

Chlorophyll *a* fluorescence parameter F_v/F_m and *Performance index* (P_{Index}) in barley and lettuce leaves changed differently under impact of Pb pollution. Significant decrease in F_v/F_m values was found at the end of the experiment for both model plants. Thus, on the 28th day of the experiment chlorophyll *a* fluorescence parameter F_v/F_m for barley of all Pb treatments were significantly lower than that of the control plants (Figure 4, A) and ranged from 0.775 to 0.796 (0.811 for the control plants). Similarly, on the last day of the experiment the chlorophyll *a* fluorescence parameter F_v/F_m for lettuce leaves with different Pb level in substrate were significantly lower in comparison to control plants (0.829) and ranged from 0.763 to 0.797(Figure 5, A).

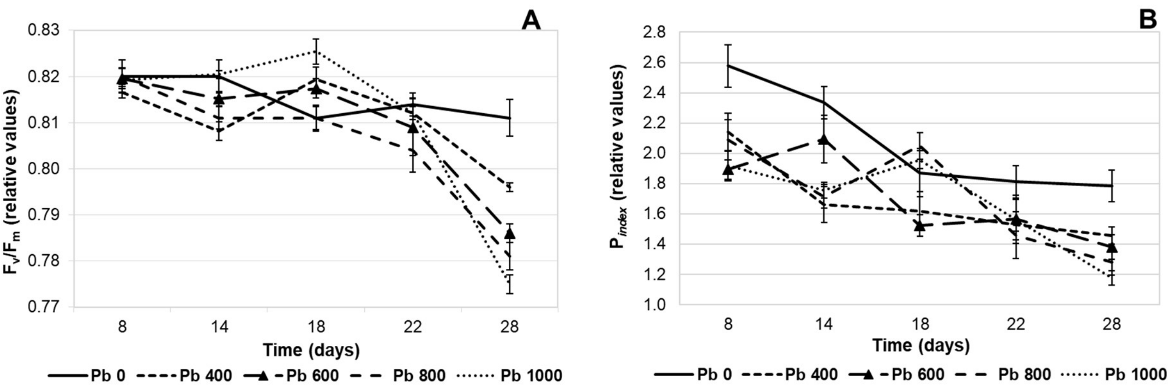

Figure 4. Chlorophyll *a* fluorescence parameter F_v/F_m (relative values) (A) and P_{Index} (relative values) (B) in *H.vulgare* L. leaves at five levels of Pb added in substrate, ±SE

There were different pattern of Pb impact on *Performance index* for two model plant species studied. In general, P_{Index} showed that the total physiological vitality of barley plants with different Pb level in substrate is significantly lower than that of control plants (Figure 4, B) during the experiment. Unlike to barley, there were mostly stimulative impact of Pb pollution on P_{Index} for lettuce (Figure 5, B). The most significant changes were found on the 33rd day of the experiment, when P_{Index} for Pb treatments (250, 300 and 350 mg L^{-1}) reached higher relative values, i.e. from 2.543 to 2.602 than control plants (relative value 1.855). Only on the last day of the experiment, the P_{Index} relative value for control plants was higher than that of Pb treatment plants (Figure 5, B).

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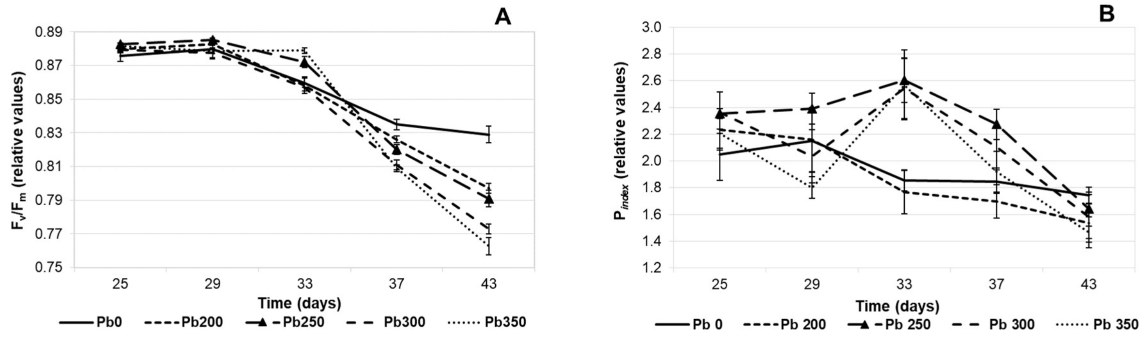

Figure 5. Chlorophyll *a* fluorescence parameter F_v/F_m (relative values) (A) and P_{Index} (relative values) (B) in *L. sativa* L. leaves at five levels of Pb added in substrate, ±SE

Plant exposure to increasing levels of Pb caused a simultaneous accumulation of this element in model plants. Close positive correlation between Pb concentration in substrate and in leaves (correlation coefficient r=0.846) and roots (r=0.776) of barley and in leaves (r=0.904) and roots (r=0.908) of lettuce was found. Pb concentrations in roots were considerably higher than in leaves of plants studied (Figure 6-7, A, B). In most of cases the highest Pb concentrations were found for second sampling time: the 14th day of the experiment for barley and the 29th day of the experiment for lettuce. Thus, under the highest Pb concentration in the substrate - 1000 mg L^{-1} for barley and 350 mg L^{-1} for lettuce - the concentration of Pb in barley leaves reached 900.60 mg kg^{-1} and in lettuce leaves reached 356.50 mg kg^{-1} (Figure 6 A, 7 A). At this sampling time and pollution levels, the concentration of Pb in barley roots was 19.32 and in lettuce roots 15.43 times higher than in the leaves.

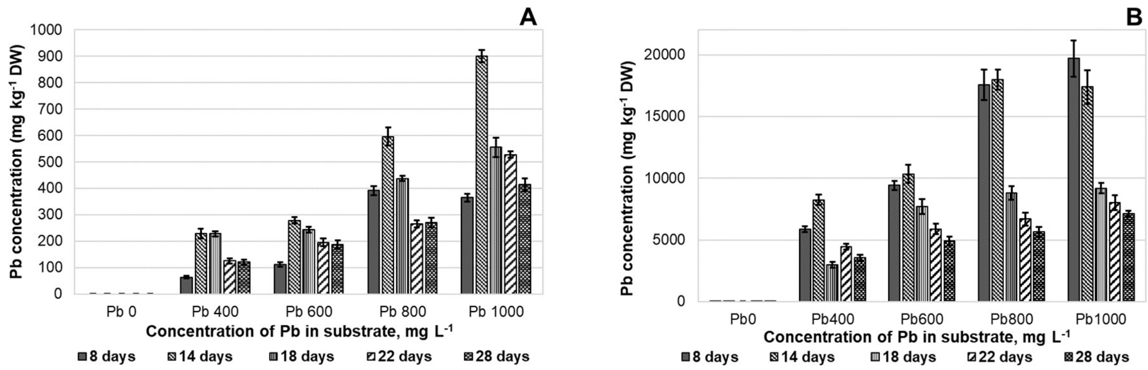

Figure 6. Pb concentrations (mg kg^{-1} DW) in the leaves (A) and roots (B) of *H. vulgare* L. at five levels of Pb added in substrate, ± SE

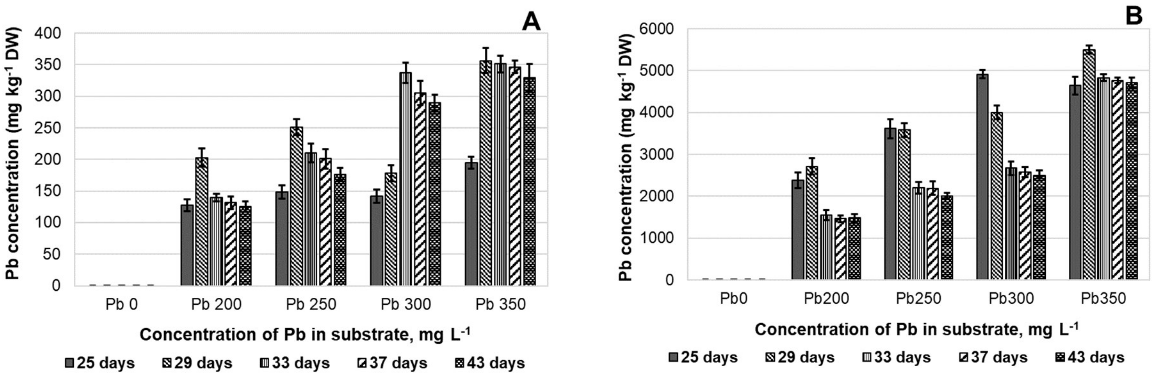

Figure 7. Pb concentrations (mg kg^{-1} DW) in the leaves (A) and roots (B) of *L. sativa* L. at five levels of Pb added in substrate, ± SE

The effect of external Pb on nutrient – Mg, Mn and Fe - concentrations in leaves and roots of barley and lettuce is shown in Figure 8.

The obtained results confirmed, that in general the Mg concentration in barley leaves was the largest in the smallest (Pb 400 and Pb 600) treatment variants, respectively for the treatment variant Pb 400 on the 14th and 18th day of the experiment Mg concentration was 0.06% and 0.07% (1.2 times) higher and on the 22nd and 28th day of the experiment - 0.12% and 0.11% (1.4 times) higher than that of the control plants, but for the treatment variant Pb 600 on the 22nd and 28th day of the experiment - 0.11% (1.4 times) higher (Figure 8, A). Variance analysis confirmed that the concentration of Mg in barley leaves was significantly depended on the amount of Pb delivered in the substrate and time throughout the experiment (ANOVA, $p<0.05$).

Mg concentration in lettuce leaves of all treatment variants plant was higher than that in the control plants throughout the experiment (Figure 8, B). On the 33rd, 37th and 43rd day of the experiment, Mg concentration in lettuce leaves of all Pb treatments was higher than that of the control plants leaves. Thus, at the last day of the experiment, the concentration of Mg in lettuce leaves of treatment Pb 200 was 0.39 % (1.55 times higher than that of the control), Pb 250 - 0.40 % (1.57), Pb 300 - 0.30 % (1.19) and Pb 350 - 0.33 % (1.29). The Pb effect on Mg concentration in lettuce leaves was significant at the beginning and the end of the experiment (ANOVA, $p<0.05$).

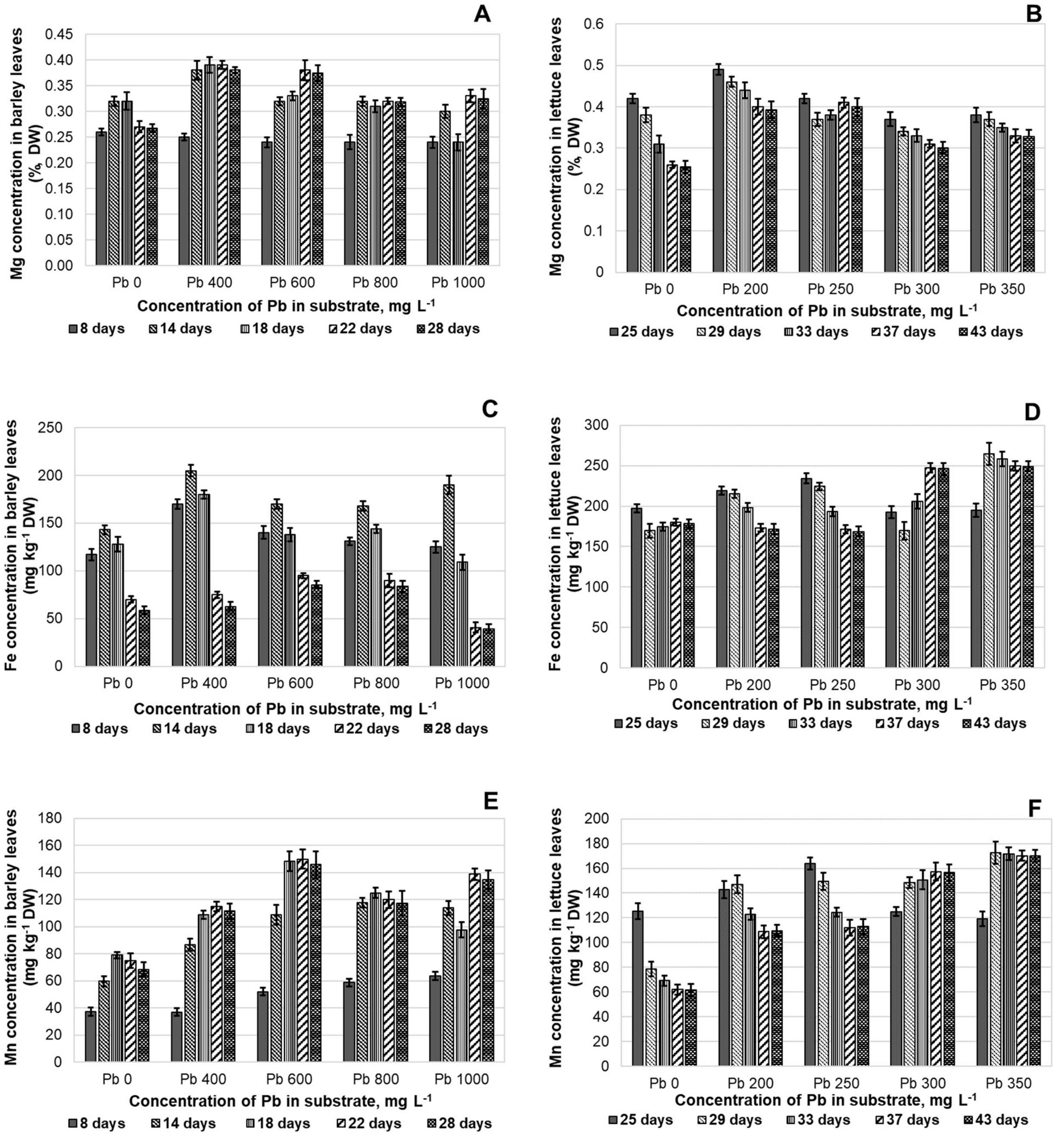

Figure 8. Mg concentration (%, DW) and Fe, Mn concentrations (mg kg^{-1} DW) in the leaves of *H. vulgare* L. (A, C, E) and *L. sativa* L. (B, D, F) at five levels of Pb added in substrate, ± SE

The concentration of Fe in the barley leaves of both control and Pb treatments dropped rapidly on the 22nd day of the experiment if compare to that of the 18th day: for Pb 0 from 128 mg kg^{-1} to 70 mg kg^{-1} (1.83 times lower), Pb 400 - 180 mg kg^{-1} to 75 mg kg^{-1} (2.40), Pb 600 – 138 mg kg^{-1} to 95 mg kg^{-1} (1.45), Pb 800 - 144 mg kg^{-1} to 90 mg kg^{-1} (1.60), Pb 1000 -109 mg kg^{-1} to 40.6 mg kg^{-1} (2.68) (Figure 8, C). Increasing level of Pb in substrate resulted in the elevation of Fe concentration in barley leaves mainly for the second and third sampling time. In general, variance analysis revealed that Pb effect on Fe concentration in barley leaves was significant throughout the experiment (ANOVA, $p<0.05$).

Unlike to barley, there were no drastic decrease in Fe concentrations found for lettuce leaves during the experiment. Concentration of Fe in lettuce leaves varied differently depending on the Pb treatments (Figure 8, D). At the beginning of the experiment an increase in Fe content was found for treatments with applied Pb concentrations up to 250 mg L^{-1}. In conditions of higher pollution (Pb 300-350 mg L^{-1}), such stimulative impact on Fe accumulation in lettuce leaves was found for the end of the experiment. Therefore the concentration of Fe in lettuce leaves depended on the amount of Pb delivered in the substrate significantly throughout the experiment (ANOVA, $p<0.05$).

During the experiment increased Mn accumulation was found in barley leaves for all Pb treatments (Figure 8, E). The highest concentration of Mn in barley leaves was reached in treatment Pb 600 on the 18th, 22nd and 28th day of the experiment, respectively, 148.4 and 150 and 145.9 mg kg^{-1}. The stimulative impact of Pb on Mn concentrations in barley leaves was significant throughout the experiment (ANOVA, $p<0.05$).

A significant increase in the content of Mn was also detected in the lettuce leaves for all Pb treatments (Figure 8, F) from the second sampling time on 29th day of the experiment. In general, the concentration of Mn in lettuce leaves depends on the amount of Pb delivered in the substrate throughout the experiment (ANOVA, $p<0.05$).

Conclusions and discussion

One of the factors that strongly affects crop plants are heavy metals, including Pb. Changes in plant growth and morphology are one of the visual symptoms that indicate stressor exposure. Although, Pb had negative impact on fresh weight and plant development for both barley and lettuce, it was more pronounced for lettuce. Most clearly this impact was expressed in treatments with the highest concentration of Pb in the substrate – for barley Pb 1000 and for lettuce Pb 300 and Pb 350. A remarkable difference in plant growth between model plants was observed under Pb stress. There were almost no Pb induced inhibition of fresh biomass for barley roots stated. Moreover, Pb pollution in substrate caused significant increase on root growth, especially at the beginning of the experiment. A lack of consistent adverse effects exerted by Pb on barley roots could be considered as plant adaptive response to pollution in growing medium. It is notable that the root: shoot biomass ratio increased with increasing pollution level for both lettuce and barley. However, this increase was more pronounced for barley. The root: shoot ratio of barley and lettuce at the highest pollution levels were 1.71 and 0.74, respectively, that comprise 216% and 180% of that of the control plants. A consequent increase in the lettuce and barley root: shoot mass ratio in response to Pb toxicity indicated on greater inhibition of plant aboveground parts than roots.

The obtained results convincingly proved that Pb as non-mobile element was mainly accumulated in the roots of lettuce and barley. This is consistent with the studies of Małkowski et al. (2005) and Capelo et al. (2012) who found considerably higher concentrations of Pb in roots of *Zea mays* L. and *Lactuca sativa* L. Previous studies suggested key mechanisms that prevent transport of Pb from the roots to the plant upper parts including mechanical barriers in roots of plants (for example, endodermis of roots, and development of peri-endodermis), mobilization of the different protective mechanisms in plants, complexing, precipitation reactions of lead as Pb-phosphate in roots, development of effective root mass, etc. (Zelko et al, 2008; Pourrut et al, 2011; Capelo et al, 2012; Líška et al, 2016). In addition, there are significant differences among the plant species in the ability to accumulate heavy metals. Our results showed that in the conditions of the highest Pb concentrations in the substrate respectively 1000 mg L^{-1} for barley, the concentration of Pb in barley leaves was 414.20 mg kg^{-1}, while three timed lower pollution level for lettuce (Pb 350 mg L^{-1}), caused almost similar Pb content in lettuce leaves - 329.74 mg kg^{-1} and drastic decrease in leaf biomass at the end of the experiment. Therefore, the roots of barley as monocotyledon acted as a physiologically active protection barrier preventing the transmigration of the Pb to the aboveground parts. In addition, changes in barley biomass allocation occurred in favour of the roots starting from the concentration Pb 600 - the root : shoot ratio 1.26, while for control treatment 0.79 only. The amount of Pb accumulated in the roots compared to leaves is proportionally larger in barley than in lettuce at the last day of the experiment (for barley: Pb 400 - 29.37 times, Pb 600 - 26.16, Pb 800 - 20.91, Pb 1000 - 17.20; for lettuce: Pb 200 - 11.77 times, Pb 250 - 11.40, Pb 300 - 8.61, Pb 350 – 14.26), it also explains the translocation of Pb depending on the species.

Chlorophyll content was measured in plants in order to assess the impact of heavy metal stress, as changes in pigment content are linked to visual symptoms of toxicity and plant photosynthetic productivity. In the present study, Pb pollution in substrate resulted in stunted growth and chlorosis for lettuce and barley. Although, there were differences in the pattern of Pb impact on chlorophyll $a+b$ content in barley and lettuce leaves, overall, this effect was negative. According to Seregin and Ivanov (2001) Pb was reported to disrupt photosynthesis by changed chloroplast ultrastructure, reduced synthesis of chlorophyll, plastoquinone, carotenoids, disturbed electron transport, inhibited enzyme activities of the Calvin cycle, caused CO_2 deficiency due to stomatal closure. Reduction of chlorophyll content in the conditions of Pb contamination may also occur due to inhibition of chlorophyll – synthesizing enzymes (Seregin and Ivanov, 2001; Cheng, 2003; Yadav, 2010). Our results revealed that Pb contamination in the substrate in low concentrations promoted the synthesis of chlorophyll in lettuce leaves in the middle stage (the 33rd and 37th day) of the experiment. Stimulatory impact of low Pb concentrations on the photosynthetic apparatus was found also for barley and oat (Kaznina et al, 2005).

Chlorophyll *a* fluorescence can be used as a tool to identify a specific stress, consequently, allowing to find out the effect of a stressor on the photosynthesis apparatus (Kalaji et al, 2014). It is defined that the fluorescence F_v/F_m values can vary approximately between 0.78-0.84 under optimal conditions depending on plant type - C3 or C4 plants, broadleaved or narowleaved species, algae, lichens etc. under stress conditions this values are reduced (Kalaji et al, 2014). It is assumed that the limit value for F_v/F_m between optimal and stress conditions is 0.8. At the end of the experiment, for both barley and lettuce, F_v/F_m values were below 0.8 for all Pb treatment variants, while for the control variant plants F_v/F_m values were above 0.8. This indicated on Pb as stress factor causing photoinhibition for both studied plants. It is notable that in the middle of the experiment (for barley on the 18th day and for lettuce on the 33rd day), F_v/F_m values of almost all Pb treatments increased above the control F_v/F_m values, thus indicating on activation of maximum quantum yield of PS II photochemistry. P_{Index} values, representing vitality of plants, for Pb treated barley were lower than that of the control during all the experiment (except higher treatments Pb800 and Pb1000 on the 18th day of the experiment) thus, showing Pb as a stress factor. On contrary, P_{Index} values for lettuce of all treatments were less than that of the control variant only at the end of the experiment, thereby pointing out differences between plant species studied. The obtained results confirmed that changes in photosynthesis describing parameters is a species-specific response to substrate contamination with Pb. Changes of photosynthetic pigment amount and chlorophyll *a* fluorescence parameters gave evidence about possible plant resistance mechanisms to Pb as a stress factor. At the same time, plants are able to adapt to heavy metal pollution, that can result even into the activation of physiological processes.

Different studies showed that heavy metals can induce changes in the uptake of essential nutrients by plants (Siedlecka, 1995; Kabata-Pendias and Kabata, 2001; Cheng, 2003; Sharma and Dubey, 2005; Yadav, 2010; Capelo et al, 2012; Kaur et al, 2013). Our studies show the ability of one element to inhibit or to stimulate the uptake of other elements was clearly demonstrated under increasing loads of Pb for both lettuce and barley. In general, Pb caused an increase of the Mg, Fe and Mn concentrations in lettuce and barley leaves. It is notable that this increase was already observed with low Pb pollution levels in substrate. There was a certain relationship between the increase of nutrient concentrations and changes in photosynthetic pigment content and/or chlorophyll *a* fluorescence parameters, which also tend to increase in the middle of the experiment for lettuce and barley. As Mg, Mn and Fe are directly involved in processes of photosynthesis (Marschner, 1995; Sengar et al, 2008; Pourrut et al, 2011), this increase could be considered as a plant adaptive response aimed to protect or activate photosynthesis in the conditions of Pb toxicity. Different previous studies on alterations in nutrient content caused by Pb stress reported mostly on the reduction of essential macro and micronutrients in the wide range of plant species (Siedlecka, 1995; Pourrut et al, 2011; Capelo et al, 2012). Controversially, there are several evidences on the increase in Mn concentration for spinach and wheat (Lamhamdi et al, 2013), Mn and Fe – for tomato (Khan and Khan, 1983) under Pb application in substrate. As the concentration behaviour of micro- and macronutrients in plants under contamination by Pb seems to differ significantly among different species and pollution levels, additional studies are necessary for particular plant species and heavy metal stress conditions. In addition, Capelo et al. (2012) have reported that lettuce is recommended as a standard species for toxicity testing in ISO tests, so it is important to continue studies on the impact of heavy metals on food-producing plants. For a more detailed explanation of the above changes in the model objects studied, it is necessary to investigate the anatomical changes of the roots of the monocotyledons and dicotyledons.

Summarizing our results we can conclude that lettuce and barley exposure to Pb contamination resulted in massive accumulation of Pb in plants and growth inhibition. There were significant differencies in the ability of Pb accumulation between model objects showing that lettuce was more susceptible to Pb toxicity than barley. A lack of consistent adverse effects exerted by Pb on barley roots could be considered as a plant adaptive response to pollution in growing medium.

Although, overall Pb impact on parameters characterizing photosynthesis as chlorophyll content, F_v/F_m and P_{Index} in barley and lettuce leaves was negative, there was also some stimulatory effect found for these parameters giving evidence on possible plant protective reaction to Pb as a stress factor. The increase of the Mg, Fe and Mn concentrations in lettuce and barley leaves induced by Pb treatments also pointed out possible activation of photosynthesis in the conditions of Pb toxicity. Further studies are necessary to carry out on heavy metal pollution impact on photosynthesis, nutrient accumulation and anatomical changes of the roots of the monocotyledons and dicotyledons to reveal tolerance mechanisms for crop plants.

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Summary

INFLUENCE OF LEAD ACCUMULATION ON PHYSIOLOGICAL PARAMETERS AND MINERAL ELEMENT (Mg, Fe, Mn) UPTAKE IN CROP PLANTS

Vita Alle, Anita Osvalde, Uldis Kondratovics, Mara Vikmane

Soil pollution with hazardous elements including heavy metals has become a problem all around the world. Heavy metals can cause health problems for people through access to the food chain. The aim of this study was to find out the impact of different lead (Pb) concentrations in the substrate on Pb accumulation in barley and lettuce leaves and roots, uptake of mineral elements, as well as effect on photosynthesis and chlorophyll *a* fluorescence, thus revealing differences between monocotyledons and dicotyledons. Plants were grown up in quartz sand under controlled growth conditions and under increasing level of Pb in substrate. There were differencies in the ability of Pb accumulation between model object leaves during the experiment. In general, the uptake of several mineral elements (Mg, Fe, Mn) in lettuce and barley increased under Pb pollution. An increase of Pb concentrations in substrate resulted in the decreased content of chlorophyll *a+b* in leaves of model objects. The results showed that increasing Pb concentrations in substrate has a different effect on photosynthesis describing parameters in monocotyledons and dicotyledons. Both exclusion and tolerance strategies operate as plant resistance mechanisms to Pb as a stress factor in the model objects – barley and lettuce.

Keywords: dicotyledon, heavy metals, monocotyledon, photosynthesis parameters

Data about the author:

Vita ALLE. Mg. biol, University of Latvia, Faculty of Biology, 1 Jelgavas Street, Riga, Latvia, LV-1004.
E-mail: vita.alle@lu.lv
Anita Osvalde, University of Latvia, Institute of Biology, Laboratory of Plant Mineral Nutrition, 3 Miera Street, Salaspils, Latvia, LV-2169
Uldis Kondratovics, University of Latvia, Faculty of Biology, 1 Jelgavas Street, Riga, Latvia, LV-1004.
Mara Vikmane, University of Latvia, Faculty of Biology, 1 Jelgavas Street, Riga, Latvia, LV-1004.