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Cadmium impact on the phytotoxicity of lead to spring barley *Hordeum vulgare*

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Abstract

The aim of the study was to determine cadmium influence on the lead phytotoxicity to spring barley (*Hordeum vulgare* L.). The seedlings of *H. vulgare* were treated with single Pb (ranging from 0.1 to 100 mg l^{-1}) and Pb mixture with Cd. Plant biomass production, physiological response, induction of oxidative damage and metals accumulation in roots and shoots were evaluated. Single Pb and under the presence of cadmium impaired the growth of *H. vulgare*, altered the content of photosynthetic pigments and induced lipid peroxidation. The addition of Cd to the Pb treatments has led to additive or synergistic effects on *H. vulgare* shoot and root dry weight, oxidative damage and increased bioaccumulation. The interactive effect of these metals on the content of photosynthetic pigments was concentration range dependent. Additivity was detected when *H. vulgare* were exposed to low concentrations of Cd and Pb and high concentrations of these metals had lower than additive effect.

Keywords: Accumulation, barley, cadmium, interaction, lead, oxidative stress.

1. Introduction

Heavy metals pollution of soils is considered as one of the most serious problems worldwide because these elements are toxic, not biodegradable and can be incorporated into the food chain. Heavy metals have received particular attention in toxicity studies analysing their effects on plants, though the majority of these studies focus on the possible adverse effects of single metal exposure, not mixtures (Clemens, 2006; Kopittke *et al.*, 2010; Gallego *et al.*, 2012). Since agricultural plants are simultaneously exposed to various pollutants, such as pesticides, heavy metals, fertilizers etc., it is important to investigate the combined effects of pollutants mixtures impact on agricultural plants.

Lead (Pb) and cadmium (Cd) are non-essential metals and they could be found in relatively large amounts in agricultural soils. The main sources of these metals are mining, industrial processes, atmospheric deposition, fertilizers and pesticides application. Pb is non-redox active metal and can bind strongly to amino acids, enzymes, DNA and RNA and it inhibits root growth, photosynthesis, disturbs water balance (Larbi *et al.*, 2002; Sharma and Dubey, 2005). Lead is extremely phytotoxic and the median toxic concentration is c. 1 μM (Kopittke *et al.*, 2010). Despite low Pb solubility and strong interaction with soil particles, elevated Pb levels are usually recorded in plants growing near roads or in industrial areas (Shadid *et al.*, 2017). Cd is also strongly phytotoxic and causes severe biochemical, physiological and morphological effects. Cd inhibits the growth, alters the functionality of membranes, interferes with enzymatic activities related to photosynthesis, disturbs nutrient translocation in plants, induces oxidative stress (Sandalio *et al.*, 2001; López-Millán *et al.*, 2009).

As Cd and Pb are usually found simultaneously in the soils and they could be uptaken by plants as divalent cations (Roth, 2006; Sharma and Dubey, 2005), it could lead to joint effects. Metals mixtures were shown to have more profound effects compared to the toxicity observed at exposure to single metal (Versieren *et al.*, 2016). Scientific and regulatory concern over the ecological effects and risks assessment of pollutant mixtures has increased within the last several decades, though the current regulatory risk assessment of chemicals is still based on single toxicity data (Backhaus and Faust, 2012). Joint effects of metal mixtures can result in effect additivity, synergism and antagonism. Additivity often occurs when the components of the mixture affect the same target or have the same mode of action. Synergism may occur due to accelerated bioaccumulation, inhibition of detoxication of one of the component of the mixture or due to increased bioactivation of one of the component of the mixture. In case of antagonism, the observed mixture effect level is lower than predicted by summing the effects for the individual components of the mixture. The predominant responses of metals mixtures were reported to be antagonism and synergism, irrespective of the organisms and environmental compartment (Vijver *et al.*, 2011). The effects of metals mixtures on aquatic and terrestrial plants have been studied extensively, especially Cd mixtures with essential metals (such as Cu, Zn) (Versieren *et al.*, 2017; Horvat *et al.*, 2007; Qian *et al.*, 2009; Yan *et al.*, 2015). Montvydienė and Marčiulionienė (2004) have observed that concentrations of Cd, Cu, Cr, Mn, Pb, Zn and Ni in mixtures that caused the same adverse effects to *Lepidium sativum* and *Spirodela polyrrhiza* were lower than in single treatments and sometimes the differences reached two orders of magnitude. Interactive effects of non-essentials Cd and Pb on crop growth were not analysed while additive or synergistic effects were recorded in aquatic plants (Alonso-Castro *et al.*, 2009; Saygideğer, Doğan, 2004). Though different cadmium and lead bioavailability from environment and similar pathway of uptake may lead to interaction of these metals and joint toxicity could be different compared to single metal toxicity (Clemens, 2006; Sharma and Dubey, 20025). The aim of the study was to determine how cadmium addition can influence lead effects to spring barley (*Hordeum vulgare* L.). Plant biomass production, physiological response, induction of oxidative damage and metals accumulation were evaluated.

2. Material and methods

Spring barley (*Hordeum vulgare* L.) after seed sterilization was germinated on moisture filter paper in dark at 20 ± 1 °C for 3 days. After germination seedlings were grown for five days in hydroponics filled with aerated nutrient solution (0.4 mM $CaCl_2$, 0.65 mM KNO_3, 0.25 mM $MgCl_2*6H_2O$, 0.01 mM $(NH_4)_2SO_4$, 0.04 mM NH_4NO_3). Seedlings were exposed for 5 days to single lead (as PbSO4), the tested concentrations were 0.1, 1, 5, 10 and 100 mgPb l^{-1}. According to the same procedures the experiment with the addition of Cd (as $CdSO_4{\cdot}8/3H_2O$) was executed. The treatments concentrations were 0.1Pb + 0.1Cd, 1Pb + 1Cd, 5Pb + 5Cd, 10Pb + 10Cd and 100Pb + 100Cd, in mg l^{-1}. Three replicates for each single and joint heavy metal treatment and control were used. The range of the tested metal concentrations was chosen to simulate the levels of these metals as they are found in soil – from uncontaminated, slightly contaminated to severely contaminated (Reimann *et al.*, 2012). The similar Cd concentrations were tested in other previous studies (Versieren *et al.*, 2017; Žaltauskaitė and Šliumpaitė, 2013ab). The plants during the experiment were from 9th to 11th growth stage according to BBCH (Meier, 2001). Experiments were carried out in controlled chambers: photoperiod – 14 hours, temperature – 22 ± 1°C at daytime and at 16 ± 1°C at night, relative humidity – 65%, light intensity of 14000 Lx.

The following endpoints were measured: plant growth as dry weight, root length, content of photosynthetic pigments (chlorophyll a, b, carotenoids), content of malondialdehyde and metal bioaccumulation. Single Cd toxicity to *Hordeum vulgare* was discussed in previous our paper (Žaltauskaitė and Šliumpaitė, 2013a).

Content of chlorophylls (a, b) and carotenoids was measured spectrofotometrically in 100% acetone extract (von Wettstein, 1957). Concentration of malondialdehyde (MDA), was used as biomarker of membrane oxidative damage. MDA content was determined by reaction with thiobarbituric acid (Buege and Aust, 1978).

Bioaccumulation of metals was measured in plant roots and shoots. Plant samples were dried for 48 h at 70°C temperature and digested using Milestone Ethos One closed vessel microwave system. Metals concentrations were determined with Shimadzu AA-6800 atomic absorption spectrometer.

Metal interaction in the mixtures was assessed using Abott's formula (Gatidou and Thomaidis, 2007). The expected inhibition of the mixture, expressed as percent C_{exp}, were predicted as follows:

$$C_{\exp} = A + B - \left(\frac{AB}{100}\right) \qquad (1)$$

where A and B are the inhibitions caused by the single metals.

The ratio of inhibition (RI) was calculated as follows:

$$RI = \text{observed inhibition}/C_{exp} \qquad (2)$$

Interaction type was evaluated by comparing RI with 1. RI values > 1 indicated synergism; R = 1 – additivity, R < 1 – antagonism.

A one-way analysis of variance (ANOVA) was used to assess the concentration effect on estimated endpoints. Significant differences between control and treatments were determined by the Student's t-test and $p < 0.05$ were considered to be significant. Least Significant Difference (LSD) tests were used to evaluate statistically significant differences between the treatments.

3. Results and discussion

Exposure to single Pb had no significant impact on the dry weight of *H. vulgare* shoot (ANOVA, $F = 0.72$, $p = 0.62$) (Figure 1). The inhibitory effect of Pb on the root dry weight was observed only at the highest Pb concentration where the dry weight of root was by 43.25 % lower than in the control. Single Cd impact was more severe to dry weight of *H. vulgare* (Žaltauskaitė and Šliumpaitė, 2013a). Negative impact of Pb on plant biomass was recorded in several studies, though the impact usually could be denoted as moderate. Lead concentrations up to 2 mM had no significant effect on shoot and root dry weight of *Beta vulgaris* (Larbi *et al.*, 2002). Significant reduction in shoot biomass production was reported for *Sesbania drummondii* after exposure to 250 mg l^{-1} of Pb (Israr *et al.*, 2011).

The presence of Cd in the solution resulted in the significant inhibition of shoot and root dry weight (ANOVA, $F_{shoot} = 18.47$, $F_{root} = 6.35$, $p < 0.01$). The dry weight of shoots in the treatments with Pb + Cd was by 21.31-68.30 % ($p < 0.05$) lower than that of controls and by 24.84-53.97 % lower than that of single Pb treatments. The root dry weight was reduced by 16.34-65.31 % after the exposure to the mixture of Pb and Cd. The results indicated that Cd amplified the negative impact of Pb to weight increment and root dry weight has reached up to

87.23% of root dry weight after single treatment with Pb. The addition of Cd to the Pb treatments has led to additive or synergistic effects on *H. vulgare* shoot and root dry weight (RI > or = 1).

Similar Pb interactions with other heavy metals were shown in other studies. Israr *et al.* (2011) found that single Pb treatment (250 mg l^{-1}) was less harmful to perennial shrub *S. drummondii* biomass production than Pb mixtures with Cu, Ni and Zn. Binary mixture of Cd and Pb had additive interaction on *Cucumis sativum* shoot growth and greater than additive interaction for root growth (An *et al.*, 2004). Greater Pb phytotoxicity under the presence of Cd was also determined to *Typha latifolia* (Alonso-Castro *et al.*, 2009).

Pb had a significant effect on the concentrations of photosynthetic pigments (ANOVA, $F_{chl\ a}$ = 11.01, $F_{chl\ b}$ = 5.91, F_{car} = 3.83, $p < 0.05$) (Figure 2). The responses of both, chlorophyll a and chlorophyll b, were of the same pattern. Concentrations of chlorophyll a and chlorophyll b decreased along with Pb concentrations in the solution ($R^2_{chl\ a}$= 0.56, $R^2_{chl\ b}$= 0.67, $p < 0.05$). Pb concentrations up to 10 mg l^{-1} had minor effect on the concentrations of carotenoids and only in the treatment with 100 mgPb l^{-1} the concentrations of carotenoids were significantly reduced ($p < 0.05$).

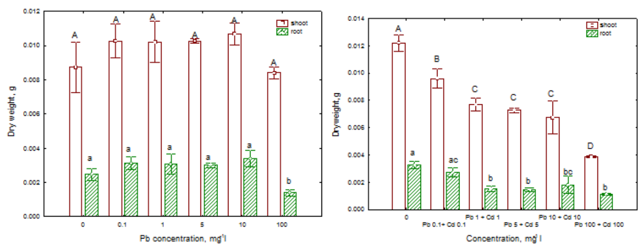

Figure 1. Spring barley *H. vulgare* shoot and root dry weight after exposure to single Pb and mixture of Pb and Cd. Different letters (capital letters – shoot, small letters – root) above the columns indicate significant difference ($p < 0.05$) between treatments (LSD test)

The presence of Cd in the solution reduced negative impact of Pb on the concentrations of photosynthetic pigments and no significant impact of Pb + Cd mixture was found (ANOVA, F < 0.63, $p > 0.05$). The concentrations of photosynthetic pigments in barley treated with both, Pb and Cd, were only marginally lower than controls. Abotts's formula calculations have showed that addition of Cd to the Pb treatment had additive or even antagonistic interaction. Additivity (RI did not differ from 1) was attributed to the mixtures composed of low Cd and Pb concentrations (0.1-1 ml l^{-1}) when addition of Cd has led to a higher impact on the chlorophyll a, b and carotenoids content. Whereas the effects of the mixtures composed of high Cd and Pb concentrations were antagonistic (RI < 1) and it may be presumed that Cd has mitigated negative Pb effects on the content of photosynthetic pigments.

Only moderate effects of Pb on the physiological characteristics of other agricultural plants were shown in other studies. Pb (10 µM – 2 mM) had very small effects on the concentrations of photosynthetic pigments in *Beta vulgaris* (Larbi *et al.*, 2002).

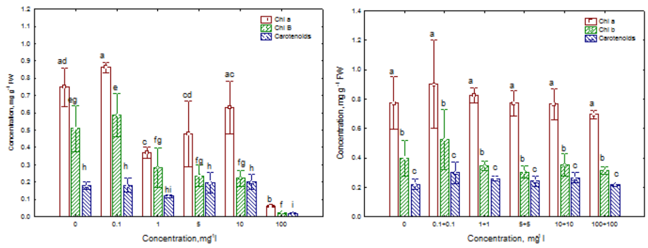

Figure 2. Concentration of photosynthetic pigments (chlorophyll a (chl a), chlorophyll b (chl b), carotenoids) in the leaves *of H. vulgare* exposed to single Pb and mixture of Pb and Cd. Different lowercase letters above the columns indicate significant difference ($p < 0.05$) among the treatments (LSD test), each parameter was evaluated separately

Pb is non- redox active metal, though it can bind to various enzymes, amino acids and mediate the accumulation of reactive oxygen species and thereby induced lipid peroxidation and oxidative stress (Wang *et al.*, 2008b). Exposure to single Pb and with Cd addition has significant effect on MDA concentration in the cells (ANOVA, F_{Pb} = 3.54, F_{Pb+Cd} = 4.01, $p < 0.05$) (Figure 3). Our results revealed that the concentrations of MDA were slightly higher when barleys were exposed to mixture of Pb and Cd compared to the levels of MDA after single Pb exposure. Low levels of Pb (0.1-1 mg l^{-1}) did not induce the lipid peroxidation though the presence of Cd in the solutions led to a slightly increase in MDA content. The increase in MDA after single Pb exposure was observed only in the treatments with 10-100 mgPb l^{-1}, though single Cd exposure had a significant effect from 10 mgCd l^{-1} (Žaltauskaitė and Šliumpaitė, 2013a). Exposure to combine mixture of Pb and Cd with 10-100 mg l^{-1} of each metal in the solution has led to a significant increase in MDA level, 1.87 and 2.52 times, respectively. The induction of lipid peroxidation and MDA accumulation after exposure to single Pb previously was recorded in tomato and *Vicia faba* (Wang *et al.*, 2008ab). Higher MDA content after the treatment with Cd imply that these two metals exhibit more than additive effect on the lipid peroxidation and it may lead to higher oxidative damage.

The content of lead in *H. vulgare* roots and shoots was significantly affected by Pb concentrations (ANOVA, $p < 0.05$) (Figure 4). Pb accumulation in roots was up to 10.36-fold higher than in shoots and increased along with Pb concentration in the medium (R^2 = 0.64, $p < 0.01$). The accumulation of Pb in the roots was affected by the presence of Cd in the solution resulting in the increase in the accumulation of Pb at the range of high exposure concentrations and slight decrease at the low concentration range. Pb bioconcentrations in the roots increased along with Pb concentrations in the mixture of Pb and Cd (R^2 = 0.89, $p < 0.01$).

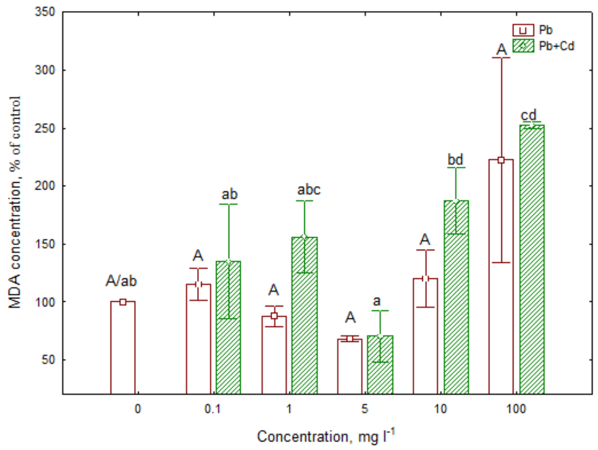

Figure 3. Concentration of MDA in the leaves tissues of *H. vulgare* exposed to single Pb and mixture of Pb and Cd. Different letters (capital letters – Pb, small letters – Pb + Cd) above the columns indicate significant difference ($p < 0.05$) between treatments (LSD test)

The translocation of Pb from roots to shoots have reached up to more than 60 %. Higher translocation of Pb was detected in the treatments with single Pb (t-test, $p < 0.05$) and translocation of Pb decreased with external metal concentration. The influence of Cd on the bioconcentrations of Pb in shoots was analogous as that in the roots, though the difference between Pb accumulated from the solutions containing single Pb and the solutions with both metals were somewhat lower. It implies lower Pb translocation when plants were exposed to a combination of Cd and Pb. Low Pb mobility from roots to shoots was also shown in the study conducted by Yoon *et al.* (2006) with different 17 terrestrial plant species and none of the plant species accumulated Pb above 1000 mg kg^{-1} in the shoots, the criteria for a hyperaccumulator (Baker and Brooks, 1989). Larbi *et al.* (2002) have found that *Beta vulgaris* exposed to Pb in the solution had taken only 0.2% of the total Pb in nutrient solution. However, *Salix viminalis* seedlings grown in sewage sludge amended soil tended to accumulate higher concentrations of Pb in leaves than in roots (Žaltauskaitė *et al.*, 2017).

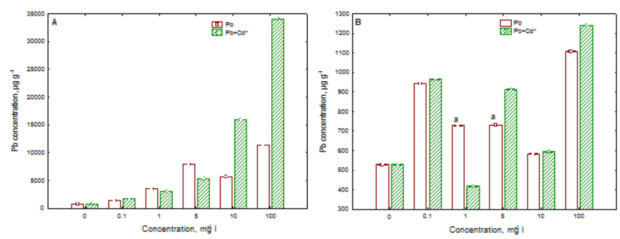

Figure 4. Lead accumulation in roots (A) and shoots (B) of *H. vulgare* exposed to single Pb and mixture of Pb and Cd. * – concentrations – 0.1Pb + 0.1Cd, 1Pb + 1Cd, 5Pb + 5Cd, 10Pb + 10Cd and 100Pb + 100Cd mg l^{-1}. A – significant difference was found between all the treatments, B – same lsmall letters above the columns indicate nonsignificant difference ($p < 0.05$) between treatments (LSD test)

The higher Pb accumulation in *H.vulgare* shoots and roots under the presence of Cd in the solution are in accordance with the data of biomass production (Figure 1). Cd had additive and synergistic effect on plant biomass and this may be explained by higher Pb accumulation in the plant.

Very similar or slightly higher Pb uptake under the presence of Cu + Cd was shown in *Cucumis sativum* (up to 160 mg Pb kg^{-1} soil) (An *et al.*, 2004). Our results are in accordance with the other studies showing higher Pb phytotoxicity under presence of other heavy (Mac Farlane, Burchett, 2002). However, our results contradict to the data reported by Alonso-Castro *et al.* (2009) who found that Cd diminished uptake of Pb by *T. latifolia*.

4. Conclusions

Our results indicated that the growth of *H. vulgare* was adversely affected by the single Pb and its binary mixture with Cd. Single Pb exposure had moderate effects on biomass of *H. vulgare,* strong adverse effect on the concentrations of photosynthetic pigments and induced lipid peroxidation. The present study shows that Cd influence on Pb toxicity to *H. vulgare* is endpoint depending. The addition of Cd into the solution had additive or synergistic effects on the root and shoot biomass inhibition, induction of lipid peroxidation, bioaccumulation of Pb, though the impact to the concentrations of photosynthetic depending on metal concentration had additive or antagonistic effect.

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