Evaluation of heavy metals binary metals mixtures toxicity on spring barley *Hordeum vulgare*

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Abstract

Heavy metal pollution is considered as one of the most serious problems worldwide and has significant environmental and human health impact. Cadmium and lead contamination is widespread due to their intensive use. Heavy metals are always found in the environment in the mixtures rather than as single elements. Within the last several decades scientific and regulatory concern over the ecological effects and risks assessment of chemical mixtures has increased as mixtures of chemicals usually elicit different toxicity to living organisms. The aim of the study was to investigate the effect of lead (Pb) and interactive effect of the binary mixture of lead (Pb) and cadmium (Cd) to the growth of spring barley (*Hordeum vulgare* L.). The seedlings of barley were treated with single Pb (ranging from 0.1 to 100 mg/L) and Pb mixture with Cd. Single and combined metal treatment impaired the growth of spring barley, altered the content of photosynthetic pigments and induced lipid peroxidation.

Keywords: barley, cadmium, lead, mixture

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 agricultural plants, though the majority of these studies focus on the possible adverse effects of single metal exposure, not mixtures. Since agricultural plants are 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 are often 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). Elevated Pb levels in plants are usually recorded near roads or industrial areas (Shadid *et al*., 2017). Cd is 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 (Sandalio *et al*., 2001, López-Millán *et al*., 2009).

Joint effects of pollutant 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 and the measured mixture effect is simply the sum of the expected effects for the individual toxicants. 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. When synergism is observed, the mixture effect level is higher than the sum of the predicted effects for the individual toxicants in 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.

Very few studies have investigated heavy metals mixture impact on commercial crop species (Luo and Rimmer, 1995; Peralta-Videa *et al*., 2002; An *et al*., 2004), the majority of works were done with aquatic plants and algae (Horvat *et al*., 2007; Qian *et al*., 2009). 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.

The aim of the study was to investigate the effect of lead and interactive effect of the binary mixture of lead and cadmium to the growth of spring barley (*Hordeum vulgare* L.).

2. Material and methods

Spring barley (*Hordeum vulgare* L.) after seed sterilization was germinated on moisture filter paper in dark at 20±1 ºC for 3 days. After germination seedlings were grown for five days in hydroponics filled with aerated nutrient solution (Aniol, 1984). Plants were exposed for 5 days to single lead (as PbSO4), the tested concentrations were 0.1, 1, 5, 10 and 100 mgPb/L. according to the same procedures the test with binary mixture of lead and cadmium (CdSO4•8/3H2O), the tested concentrations were 0.1Pb+0.1Cd, 1Pb+1Cd, 5Pb+5Cd, 10Pb+10Cd and 100Pb+100Cd, in mg/L. Three replicates for each heavy metal treatment and control were used. 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.

Single Cd toxicity to *Hordeum vulgare* was discussed in previous our paper (Žaltauskaitė and Šliumpaitė, 2013).

The following endpoints were measured: plant growth as dry weight, root length, content of photosynthetic pigments (chlorophyll a, b, carotenoids) and content of malondialdehyde (MDA).

Content of chlorophylls (a, b) and carotenoids was measured spectrofotometrically (DU 800 "Beckman Coulter") in 100% acetone extract (von Wettstein, 1957). Concentration of malondialdehyde (MDA), the end-product of lipid peroxidation, was used as biomarker of membrane oxidative damage. MDA content was determined by reaction with thiobarbituric acid (TBA) (Buege and Aust, 1978).

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.

3. Results and discussion

Exposure to single Pb had no significant impact on the dry weight of *H. vulgare* shoot (ANOVA, F =1.04, p = 0.44) (Fig. 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, 2013). 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).

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 % lower than that of controls. 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. Similar Pb interactions with other heavy metals were shown in other studies. Israr *et al*. (2011) found that single Pb treatment (250 mg/L) was less harmful to perennial shrub *Sesbania drummondii* biomass production than Pb binary 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).

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, $p < 0.05$) (Fig. 2). The response of both, chlorophyll a and chlorophyll b was 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 had minor effect on the concentrations of carotenoids and only in the treatment with 100 mgPb/L the concentrations of carotenoids were significantly reduced (p<0.05).

The presence of Cd in the solution reduced negative impact of Pb on the concentrations of photosynthetic pigments and no significant impact of binary mixture was found (ANOVA, F <0.63, p > 0.05). The concentrations of photosynthetic pigments in barley treated with binary Pb and Cd mixture were only marginally lower than

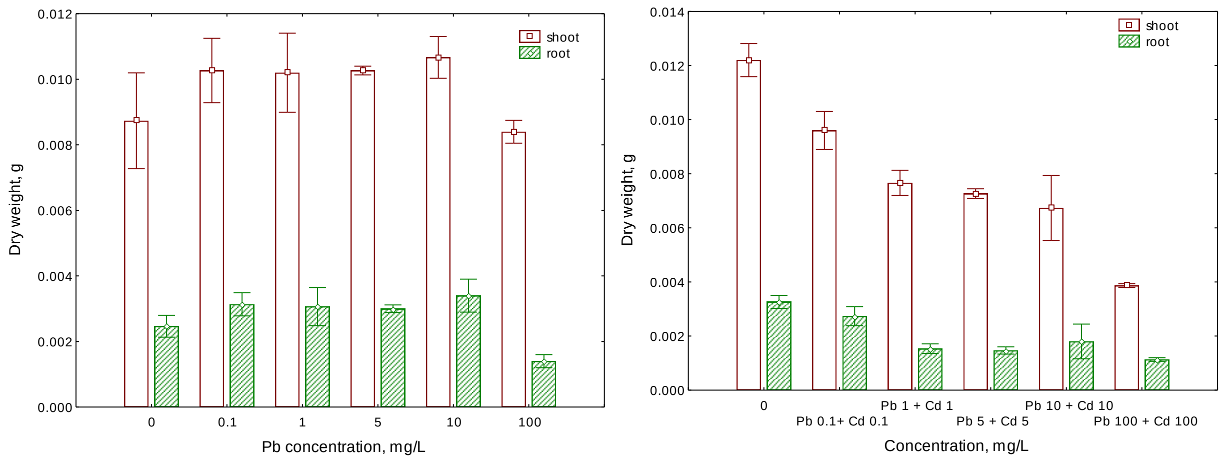

Figure 1. Barley *H.vulgare* shoot and root dry weight after exposure to single Pb and mixture of Pb and Cd

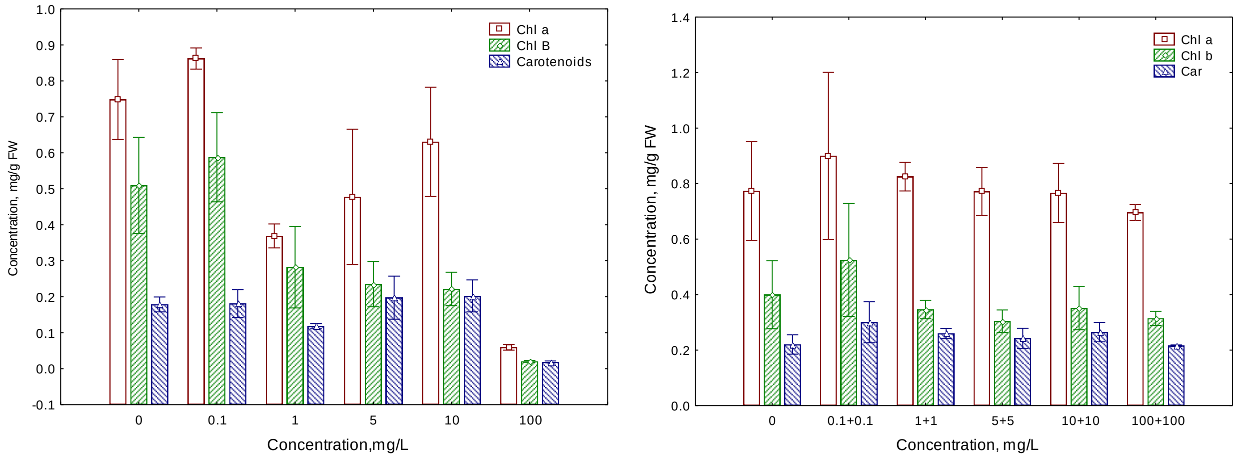

Figure 2. Concentration of photosynthetic pigments (chl a – chlorophyll a, chl b – chlorophyll b, car – carotenoids) in the leaves *of H. vulgare* exposed to single Pb and mixture of Pb and Cd

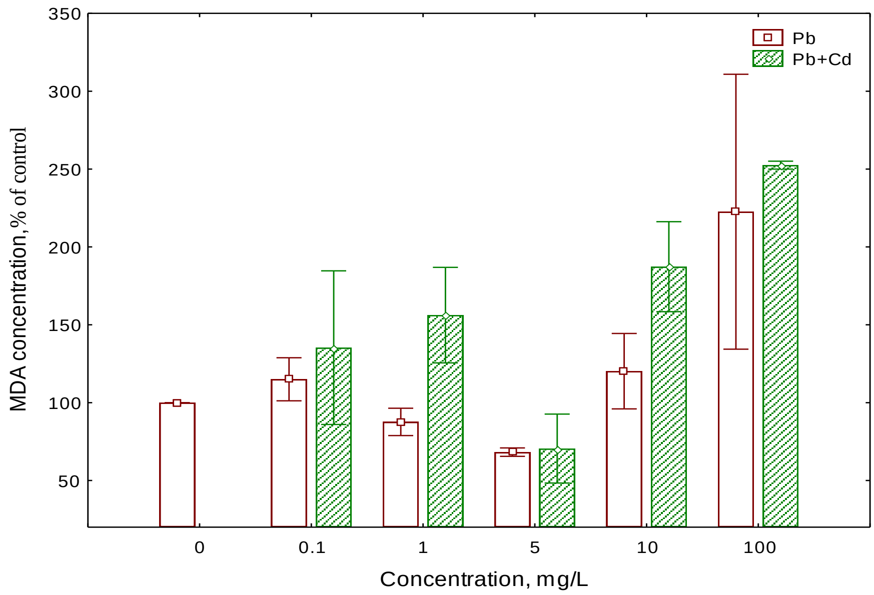

Figure 3. Concentration of MDA in the leaves tissues of *H. vulgare* exposed to single Pb and mixture of Pb and Cd

controls.It suggests antagonistic interaction between these metals.

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). The presence of Cd resulted in an enhanced reduction in H vulgare biomass compared to the exposure of a single Pb.

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 mixture of Pb with Cd has significant effect on MDA concentration in the cells (ANOVA, F_{Pb} =3.54, F_{Pb+Cd} =4.01, $p < 0.05$) (Fig. 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) 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, though single Cd exposure had a significant effect from 10 mgCd/L (Žaltauskaitė and Šliumpaitė, 2013). Exposure to combine Pb and Cd with 10-100 mg/L of each metal in the solution has led to a dramatic 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).

Conclusions

Our results indicated that the growth of *H. vulgare* was adversely affected by the single Pb and binary mixtures of Pb and 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 combined effects of Pb+Cd to *H. vulgare* are endpoint depending. The binary mixture exhibited additive or more than additive effects on the root and shoot biomass inhibition and induction of lipid peroxidation, though the impact to the concentrations of photosynthetic had less than additive impact.

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