E3S Web of Conferences **1**, 15013 (2013)
DOI: 10.1051/e3sconf/20130115013

Single and combined toxicity of copper and cadmium to *H. vulgare* growth and heavy metal bioaccumulation

J. Žaltauskaitė[1] and I. Šliumpaitė[2]

[1] Department of Environmental Science, Vytautas Magnus University, Kaunas, LITHUANIA, j.zaltauskaite@gmf.vdu.lt
[2] Department of Environmental Science, Vytautas Magnus University, Kaunas, LITHUANIA, i.sliumpaite@gmf.vdu.lt

Abstract. The single and combined effects of copper (Cu) and cadmium (Cd) (0.1-10 mg L^{-1}) in spring barley (*Hordeum vulgare* L.) plants grown in hydroponics are investigated. The aim of the study was to investigate the interactive effect of the binary mixture of Cu and Cd to the growth of *H. vulgare* and accumulation of these metals by the plants. Single and combined metal treatment led to major effects in the growth of roots and shoots and dry weight of barley. Exposure to metals altered the content of photosynthetic pigments and caused lipid peroxidation. It was observed that combined effects of heavy metals to plants are endpoint and concentration depending. The binary mixture Cu+Cd exhibited additive or less than additive interaction for dry weight, root length and shoot height. Analysis of tissue metal concentrations showed that Cu and Cd were mainly accumulated in the roots and the combination of Cu+Cd had less than additive response of metal bioaccumulation in the leaves and roots.

Key words: bioaccumulation, growth, heavy metal, *Hordeum vulgare*, mixture toxicity

Introduction

Heavy metal pollution is considered as one of the most serious problems worldwide and has significant environmental and human health impact. Copper and cadmium contamination is widespread due to their intensive industrial and agricultural use.

Cu is micronutrient, essential for plant growth in low concentrations. It constitutes plant enzymes, which trigger a variety of physiological processes in plants (photosynthesis, respiration, cell wall metabolism, etc.). Only in slightly increased concentration than required for plant growth, Cu becomes phytotoxic, reduces plant growth, disturbs nutrients metabolism, inhibits enzymes activities, causes cell damages, suppresses photosynthetic activity (Xiong et al., 2006; Martínez-Peñalver et al., 2012).

In contrast to Cu, Cd is non-essential element and is strongly phytotoxic and causes severe biochemical, physiological and morphological effects. Cd inhibits the plant growth, alters the functionality of membranes, interferes with enzymatic activities related to photosynthesis, disturbs nutrient uptake and translocation in plant (Sandalio et al., 2001, Larbi et al., 2002; López-Millán et al., 2009).

Since chemicals never occur alone in nature, and the combined effects of multiple chemicals can have a greater negative impact than does the individual constituents of the mixture, it is very important to investigate the combined effects of chemicals on living organisms. Mixture toxicity of contaminants can be classified as additive, synergistic and antagonistic. The mechanisms of mixture toxicity depend on the chemistry of mixtures compounds, their interaction in the environmental media that may influence the bioavailability, toxicological modes of action, interaction among bioaccumulated contaminants (Spurgeon et al., 2010). Interaction between heavy metals may occur at plant root surface, affecting metal uptake, and within the plant, affecting heavy metal translocation and toxicity (Luo and Rimmer, 1995). The aim of the study was to test the single and combined toxicities of copper, cadmium to spring barley (*Hordeum vulgare* L.) and to investigate the interactive effect of the binary mixture of metals.

Materials and Methods

Spring barley (*Hordeum vul*gare L. cv. Aura DS) seedlings after seed germination were grown in hydroponics filled with a half strength Hoagland's nutrient solution (Terry, 1980). Plants were exposed for 5 days to Cu and Cd separately and in combinations. The media were supplemented with different Cu (as $CuCl_2 \times 2H_2O$), Cd (as $CdCl_2$) and Cu+Cd concentrations. In single effect treatments the concentrations were 0, 0.1, 1, 5 and 10 mg L-1 of Cu (or Cd), the binary mixtures treatments were 0, 0.1+0.1, 1+1, 5+5 and 10+10 mg L-1 of Cu+Cd. 3 replicates for each treatment and control were used. Experiments were carried out in controlled chambers: photoperiod – 14 h, temperature – 22±1 °C at daytime and at 16±1 ° C at night, RH – 65%, light intensity of 14 kLx.

The following endpoints were measured: plant growth as dry weight, shoot height and root length, content of photosynthetic pigments (chlorophyll *a*, *b*), content of malondialdehyde (MDA), Cu, Cd and Fe content in leaves and roots. Content of chlorophylls was measured spectrofotometrically in 100 % acetone extract of leaves. Content of MDA was determined by reaction with thiobarbituric acid (Buege and Aust, 1978). Plant samples for Cu, Cd and Fe content determination were dried for 24 h at 70°C temperature and digested using Milestone Ethos One closed vessel microwave system. The samples were analyzed with Shimadzu AA-6800 atomic absorption spectrometer.

Binary mixture toxicity was assessed using Abott's formula (Teisseire et al., 1999). The expected inhibition of the mixture, expressed as percent C_{exp}, can be predicted as follows:

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

where A and B are the inhibitions caused by the single chemicals. The ratio of inhibition (RI) for each mixture was calculated as follows:

RI = observed inhibition / C_{exp}

A RI values > 1 indicated synergism; R = 1 – additivity, R < 1 – antagonism. If the mean RI was greater/lower than one SD from 1 (1 ±SD), the interactive effect was assumed to be significantly different from additivity.

Data were subjected to the analysis of variance (one-way ANOVA) for each endpoint. Significant differences between control and treatments were determined by Student's t-test and values p<0.05 were considered significant.

Results and Discussion

Increasing Cu and Cd concentrations in solution produced a significant impact on the shoot and root growth of *H. vulgare* (ANOVA, Cu: $F_{shoot} = 23.19$, $p < 0.001$; $F_{root} = 44.80$, $p < 0.001$; Cd: $F_{shoot} = 16.22$, $p < 0.001$; $F_{root} = 19.56$, $p < 0.001$ (Fig. 1). The growth of shoots of *H.* vulgare treated with the highest Cu and Cd concentration was inhibited by 57.39% and 48.30%, respectively. The roots were more sensitive and the length of *H.vulgare* roots, exposed to the highest concentration of Cu and Cd was 62.23% and 60.34% lower than that of control plants. The binary mixture of Cu+Cd had the significant impact on the growth of shoots and roots ($F_{shoot} = 36.23$, $p < 0.001$; $F_{root} = 37.28$, $p < 0.001$). Increasing metal concentrations in the binary solutions resulted in significant reduction of shoots height ($R^2 = 0.52$, $p < 0.001$) and the shoots exposed to the mixture of 10 mg Cu L^{-1} +10 mg Cd L^{-1} were 65.68% shorter than in control. The response of roots had slightly different pattern. The increase of concentration till 1 mg Cu L^{-1} +1 mg Cd L^{-1} resulted in sharply decrease of root length and the roots were by 38.28% lower than that exposed to 0.1 Cu L^{-1} +0.1 mg Cd L^{-1} (p<0.05) and 68.32% lower than in control (p<0.05). Further increase in metal concentration did not resulted in significant changes in root length (p>0.05).

The treatment with Cu and Cd provoked a significant impact on the dry weight of *H. vulgare* ($F_{Cu} = 23.30$, $p < 0.001$; $F_{Cd} = 10.53$, $p < 0.001$; $F_{Cu+Cd} = 11.58$, $p < 0.001$). The response of plant was very similar in case of Cu and Cd, when they were applied separately. The dry weight of *H. vulgare* treated with the highest Cu and Cd concentration in single toxicity test was inhibited by 51.87% and 47.13%, respectively.

Additivity was attributed to the mixture of Cu+Cd impact to the growth of shoots, roots and dry weight with the mean RI 0.94 ± 0.67; 0.98 ± 0.41 and 0.80 ± 0.28, respectively. Though, when the *H. vulgare* were exposed to the mixtures of > than 5 mg Cu L^{-1} +5 mg Cd L^{-1}+5, antagonistic effect was observed (RI<1). It indicates that competition between Cu and Cd may occur and it leads to lower toxicity. Keltjens and van Beusichem (1998) reported that competition between Cu and Cd may occur at common root absorption site and it results in less than additive toxicity.

Lipid peroxidation is a sensitive measure of oxidative damage and is useful as a biomarker for oxidative stress. The treatment with Cu and Cu+Cd had significant impact on MDA content in the leaves ($F_{Cu} = 134.51$, $p < 0.001$; Cu+Cd $F = 78.20$, $p < 0.001$), whereas Cd had no significant impact on MDA level ($F = 4.36$, $p=0.07$). Cu is a redox-active metal enhancing reactive oxygen species (ROS) formation via Fenton-Haber-Weiss reactions, whereas Cd, do not have redox activity, though exhibit the ability to produce ROS in the plants (Sandalio et al., 2001). MDA level doubled upon increasing the Cu concentration from 0 to 1 mg L^{-1} and quadrupled from 0 to 5 mg L^{-1}, but further increase from 5 to 10 mg L^{-1} had no significant impact on the changes in MDA content. Exposure to both Cu and Cd in the solution led to the 2.3-4.3-fold increase in MDA content. Although Cd has the small oxidative capacity, but in combinations with other metals with high oxidative capacity, may have significant impact on lipid peroxidation and it may lead to oxidative stress.

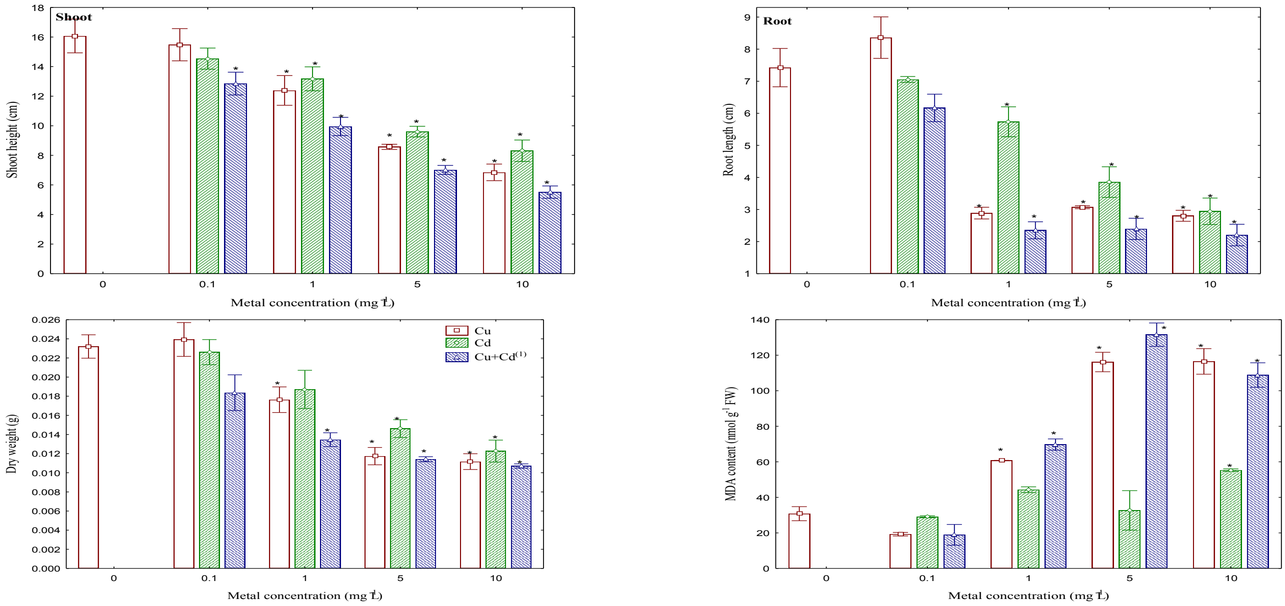

Fig. 1. Shoot height, root length, dry weigth of *H. vulgare* and MDA content in the leaves of *H. vulgare* after exposure to single Cu, Cd and in combination Cu+Cd. Data are mean ± SE. An asterisk (*) indicates significant differences ($p<0.05$) between the treatment and the control. [(1)]The concentrations in the mixture are double: 0.1 mgCu L^{-1} +0.1 mgCd L^{-1}, 1 mgCu L^{-1} +1 mgCd L^{-1}, etc.

The data of the content of photosynthetic pigment in the leaves of *H. vulgare* were very scattered and no clear relationship between the concentration of metal and chlorophyll *a*+*b* content was detected (Fig. 2). Cu had the slight stimulatory effect on chlorophyll *a*+*b* content.

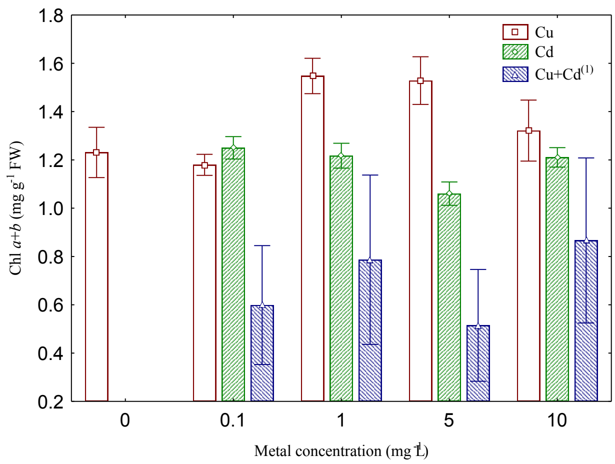

Fig. 2. The content of chlorophyll *a*+*b* in the leaves of *H. vulgare* after exposure to single Cu, Cd and in combination Cu+Cd. Data are mean ± SE. [(1)]The concentrations in the mixture are double: 0.1 mg Cu L^{-1} +0.1 mg Cd L^{-1}, 1 mg Cu L^{-1} +1 mg Cd L^{-1}, etc.

The tissue content of heavy metals was affected by single metals concentrations and increased along with metal concentration (Cu: $F_{shoot} = 5.81$, $p = 0.01$, $F_{root} = 11.38$, $p = 0.006$; Cd: $F_{shoot} = 9.73$, $p = 0.004$, $F_{root} = 22.37$, $p < 0.001$) (Fig. 3). Cu and Cd concentrations in roots were always higher than that in shoots and moreover the difference between the content in the roots and shoots after single metal treatment increased with the metal concentration in the solution. Bioaccumulation of metals was influenced by the presence of other metal in the solution resulting in the inhibited bioaccumulation. Especially this was characteristic for the concentrations in the roots. Antagonism may be explained by the fact that competition between Cu and Cd may occur at common root absorption site. Antagonistic pattern of Cu and Cd bioaccumulation is also related to the additive or less than additive toxicity of Cu+Cd mixture to the growth of roots and shoots (Fig. 1).

The exposure to single Cu or Cd and to Cu+Cd had no significant impact on Fe content in the roots of and Fe content in the leaves increased ($p<0.05$) after exposure to Cu and Cu+Cd. No effect of Cd impact on Fe content in the roots was also reported for *Pisum sativum*, while in the leaves the decrease of Fe content was observed (Sandalio et al., 2001).

Conclusion

The growth of *H. vulgare* was adversely affected by single Cu and Cd and by binary mixture of Cu+Cd. Combined effects of Cu+Cd were endpoint and concentrations depending. The mixture of Cu+Cd at low concentrations produced additive toxicity to the growth of *H. vulgare*, measured as dry weight and the growth of shoots and roots, whereas the mixture at high concentrations exhibited antagonistic impact on the growth. Bioaccumulation of metals was influenced by the presence of other metal in the solution resulting in the inhibited bioaccumulation of metals in the roots and leaves.

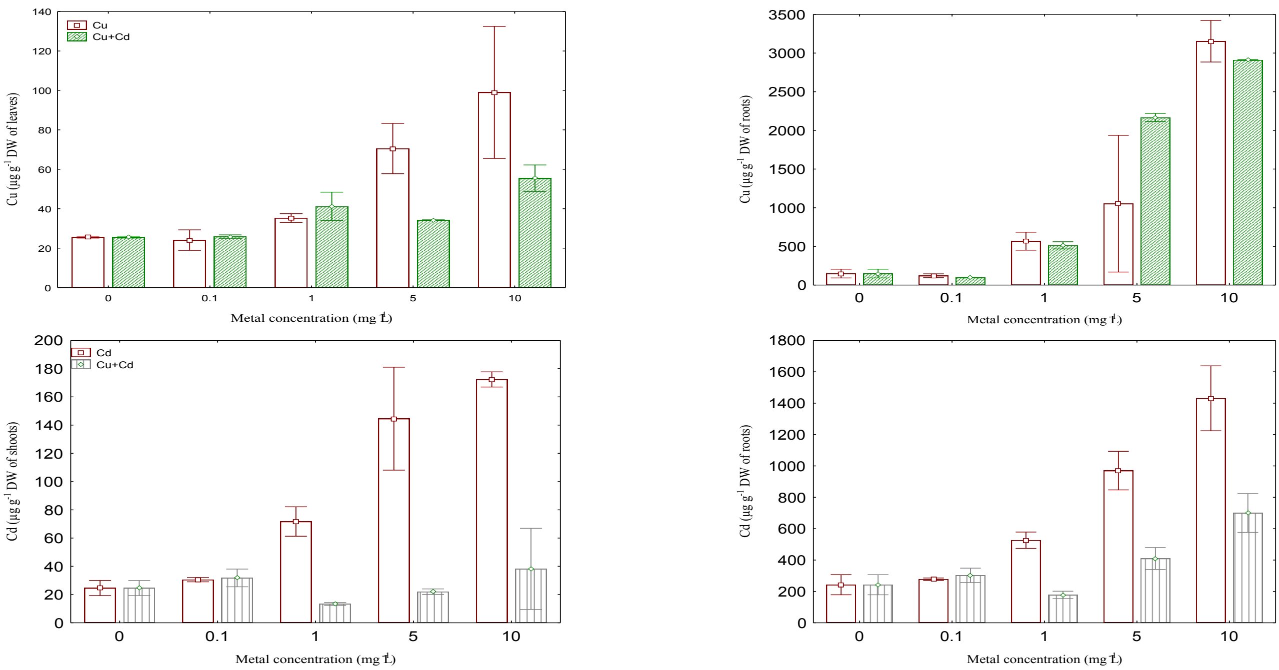

Fig. 3. Heavy metal accumulation in shoots and roots of *H. vulgare* after 5 days of exposure to Cu, Cd and Cu+Cd in combination. Data are mean ± SE.

References

Buege JA and Aust SD. 1978. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52:302-310.

Keltjens WG, van Beusichem ML. Phytochelatins as biomarkers for heavy metal stress in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.): Combined effects of copper and cadmium. Plant and soil 1998; 203:119-126.

Larbi A, Morales F, Abadía A, Gogorcena Y, Lucena JJ, Abadía J. Effects of Cd and Pb in sugar beet plants grown in nutrient solution: induced Fe deficiency and growth inhibition. Funct. Plant Biol. 2002; 29:1453-1464.

López-Millán A-F, Sagardoy R, Solanas M, Abadía A, Abadía J. Cadmium toxicity in tomato (*Lycopersicum esculentum*) plants grown in hydroponics. Environ Exper Bot 2009; 65:376-385.

Luo Y and Rimmer DL. Zinc-copper interaction affecting plant growth on a metal-contaminated soil. Environmental Pollution 1995; 88:79-83.

Martínez-Peñalver A, Graña E, Reigosa MJ, Sánchez-Moreiras AM. The early response of Arabidopsis thaliana to cadmium- and copper-induced stress. Environ Exper Bot 2012; 78:1-9.

Sandalio LM, Dalurzo HC, Gómez M, Romero-Puertas MC, del Río LA. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J Exp Bot 2001; 52:2115-2126.

Spurgeon DJ, Jones OAH, Dorne J-LCM, Svendsen C, Swain S, Stürzenbaum SR. Systems toxicology approaches for understanding the joint effects of environmental chemical mixtures. Science of the Total Environment 2010; 408:3725-3734.

Teisseire H, Couderchet M, Vernet G. Phytotoxicity of diuron alone and in combination with copper or folpet on duckweed (*Lemna minor*) Environmental Pollution 1999; 106:39-45.

Terry N. Limiting factors in photosynthesis. 1. Use of iron stress to control photochemical capacity in vivo. Plant Physiol. 1980; 65:114-120.

Xiong Z-T, Liu C, Geng B. Phytotoxic effects of copper on nitrogen metabolism and plant growth in *Brassica pekinensis* Rupr. Ecotoxicol Environ Safety 2006; 64:273-280.