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Biochemical responses of basil to aluminium and cadmium stresses

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Abstract: The effect of aluminium and cadmium on growth and physiological parameters in 60 days old basil seedlings (*Ocimum basilicum* L.) grown in polluted soil were investigated. Control plants, grown in unpolluted soil, were used for evaluation of the heavy metal effect. Growth parameters were analysed immediately after sample collection, leaf area and plant height were recorded. Analysis of secondary metabolite, proline, total carbohydrates content and antioxidant capacity in 60 days old seedlings was performed. The antioxidant activity of basil extracts was determined by the DPPH method, and results showed an increase of antioxidative capacity in basil seedlings exposed to heavy metal stress due to the activation of antioxidative system in plant cells. Maximum production of secondary metabolites was recorded for seedlings exposed to 100 mg/kg soil Cd and Al. Results showed that cadmium stress increased concentration of proline, while aluminium did not had that effect. The content of total carbohydrates remained almost unchanged, with exception of basil seedlings grown on soil containing 50mg/kg AlCl₃.

Keywords: aluminium, cadmium, basil, DPPH, secondary metabolites

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Introduction

Tolerance of plants depends on their ability to survive and reproduce under different environmental stresses (Redondo-Gomez, 2013). Nowadays one of the most serious problems for the environment is an increase of heavy metals pollutions (Golubović and Blagojević, 2012). The increased concentrations of heavy metals have a negative effect on biomass and soil fertility, but ultimately they could significantly harm human health (Gratão *et al.*, 2005).

Cadmium is a non-essential element which has a high toxicity even at low concentrations (Xu *et al.*, 2006), and in plants inhibits their growth and development, synthesis of chlorophyll, carotenoids and ATP, also causes leaf chlorosis, has influence on the function of enzymes in various metabolic pathways and decreases photosynthetic activity (Sharma and Dubey, 2006).

It is established that aluminium interferes with cell division in root tips and lateral roots, decreases root respiration, reduces accumulation of polysaccharides in the cell wall, lessens the production and transport of cytokinins as well as the water uptake, and interferes with the uptake, metabolism and transport of several crucial nutrients (Mossor-Pietraszewska, 2001).

Pollutants such as heavy metals are mainly introduced into the food chain through the root system of plants (Yadav, 2010). Homeostasis in plants is maintained by balance between the production of ROS and detoxification performed by the antioxidative system. Therefore, if the balance is disturbed by heavy metals stress, plants suffer the oxidative stress. Tolerance of stress corresponds to an increase in capacity for the removal of reactive oxygen species. Oxidative stress can lead to inhibition of photosynthesis and respiration (Sharma and Chakraverty, 2013) and production of huge number of secondary metabolites (Wink, 2010).

The higher amount of proline reduces membrane damage, regulates redox potential, decreases cell acidity, protects molecules from denaturation and provides a role of non-enzymatic antioxidants (Bacelar *et al.*, 2012). The survival of plants is closely associated with carbohydrates, respiration substrates. Morphological and physiological responses of terrestrial plants provide positive effects of carbohydrates, as well as maintaining higher concentration of carbohydrates in roots, which facilitates survival (Pradhan and Mohanty, 2013).

The aim of this work was to evaluate biochemical responses of basil (*Ocimum basilicum* L.) to Al and Cd treatments. It was studied effects of heavy metals on the content of secondary metabolites, proline, carbohydrate and antioxidant activity of basil.

Materials and methods

To perform the experiment, seeds of basil (*Ocimum basilicum* L.) were used, which were planted in the soil (previously dried for five days at room

temperature). A randomized block design experiment was arranged in the Laboratory for Plant Physiology, Department of Biology University of Sarajevo. Seeds were sown in pots (25 × 25 cm) filled with 5 kg of soil. In each pot 200 seeds of basil were planted. Soil in pots was mixed with different concentrations of aluminium chloride (Al₂Cl₃) or cadmium chloride (CdCl₂). The heavy metals concentrations were 0, 50, 100, 200 mg/kg dry soil. The pots were kept in growth chamber with 16 hours light period, temperature of 24±1°C and relative humidity of 60-80%, by watering every other day during two months. Epigeal parts were oven-dried (60°C) for 3h and used for analysis.

Dry plant material was extracted with 80% methanol, left overnight on temperature of 4°C. After centrifugation, extracts were evaporated and resuspended in 80% methanol. The radical scavenging activity of the extracts was determined using 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH•) method (Meda *et al.*, 2005) and IC₅₀ value (the concentration of extract required to inhibit 50% of DPPH free radicals) was determined. Naringenin and quercetin were applied as standard in concentration of 1mg/ml. Percentage of inhibition was compared to standard. Total phenol content was determined according to Wolfe *et al.* (2003), using the Folin-Ciocalteu reagent, and with gallic acid as standard. Analysis of total flavonoid content was performed according to Ordóñez *et al.* (2006), with catechin as standard. Analysis of total flavanols content was performed according to Gadzovska *et al.* (2007), with catechin as standard. All results were expressed as mg of used standard equivalent per mg of extract.

Extraction of proline was performed according to the modified method of Carillo and Gibbon (2001). Cold extraction process was used by mixing dry plant material with combination of ethanol : water (40:60 v/v). The supernatant was collected after the mixture was left overnight at 4°C. Quantification of proline was done by reading absorbance at 520 nm and using L-Proline as the standard.

Total carbohydrates content was determined according to Bartkiene (2012). Total carbohydrates were measured at 620 nm, using glucose as a standard.

The obtained data were analyzed statistically with ANOVA in program Statistica 8.0. Each treatment was replicated three times. Standard descriptive statistical parameters were calculated for control and all treatments. Differences between treatments were determined by Post hoc test analysis of variance using Newman-Keuls test at a significance level of 1% (P≤0.01). Calculation of the correlation coefficient by Pearson (Pearson product-moment correlation coefficient) was performed. Correlation was analyzed at the level of 5% (P≤0.05).

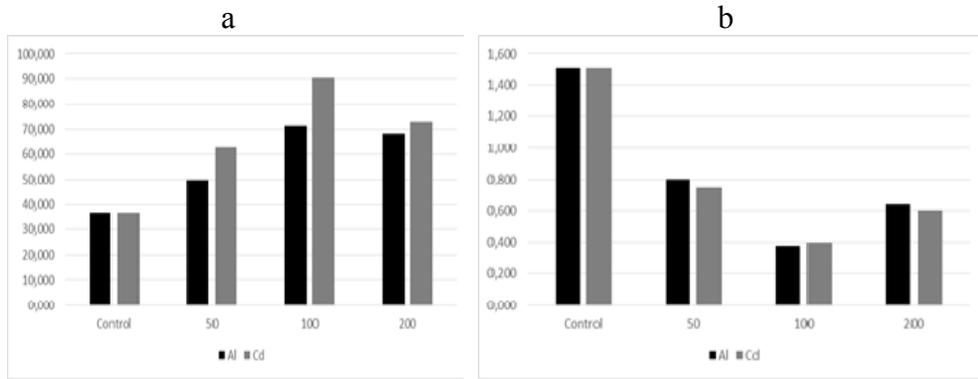


Figure 1. Percentage of antioxidative capacity (a) and IC₅₀ values (b) in basil extracts after application of aluminium and cadmium

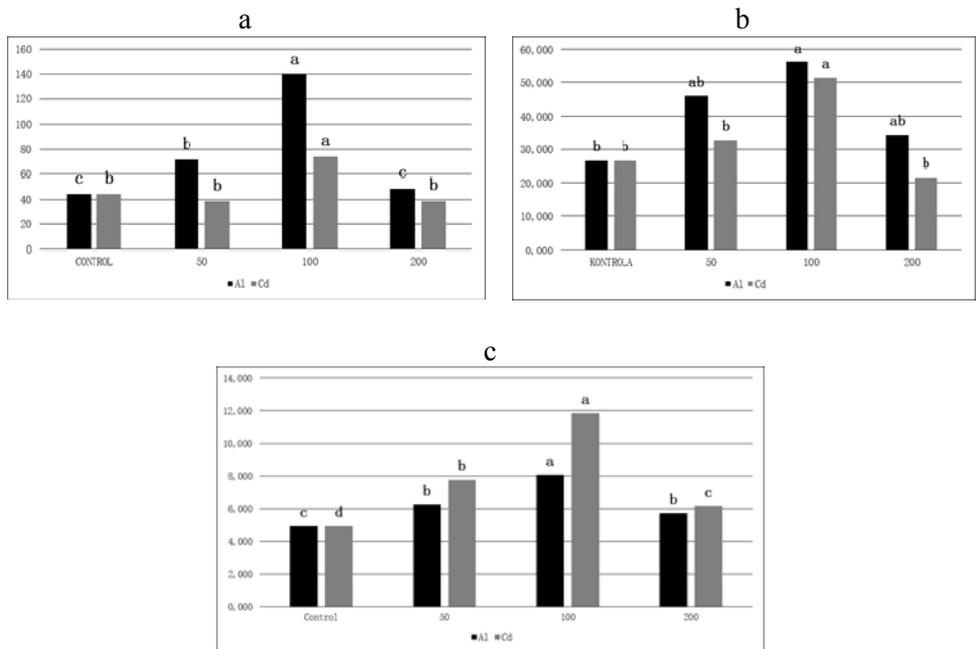


Figure 2. Variation of total phenols (a), flavonoids (b), flavanols (c) content in basil extracts after application of aluminium and cadmium

Treatments not sharing the same letter above the column differ significantly after Newman-Keuls post-hoc ANOVA test at $p < 0.01$

Results

The antioxidant capacity of methanol extracts of *O. basilicum* L., subjected to 50, 100 and 200 mg of Al and Cd was evaluated using DPPH assays. The results of antioxidant activity showed that application of aluminium and cadmium had positive effect on inhibition of DPPH radical (Fig. 1a). Almost all of analyzed treatments are found to be stronger antioxidants than the control. The presence of 100 mg Al and Cd had the highest inhibition of DPPH radicals (90.171%). The IC_{50} value was analysed for all tested treatments, and the highest IC_{50} value was registered in the control plants, indicating the lowest antioxidative activities (Fig. 1b).

The content of total phenolics, flavonoids and flavanols presented a similar pattern (Fig.2). Application of both heavy metals in concentration of 100 mg/kg soil presented the highest significant phenolic, flavonoids and flavanols content. Analysis of extracts revealed more than 50% higher content of total phenolic, total flavonoid and total flavanols contents than samples of control plants after Al treatment and about 50% after Cd treatment. And other concentration of both heavy metals increased more or less secondary metabolites content. Cd and Al stress enhanced proline content, except for the treatment of 50 mg $CdCl_2$ (Fig. 3).

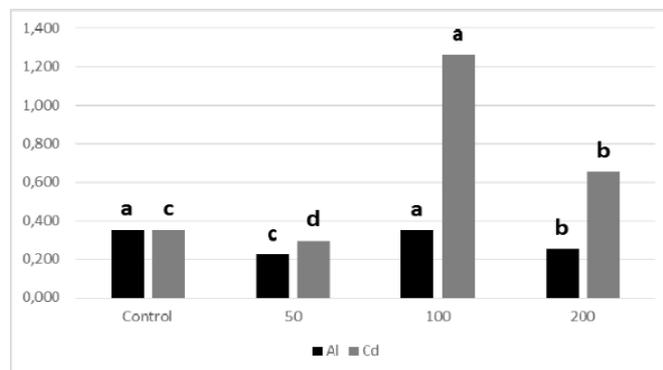


Figure 3. Variation of total proline content in basil extracts after application of aluminium and cadmium
Treatments not sharing the same letter above the column differ significantly after Newman-Keuls post-hoc ANOVA test at $p < 0.01$

From the results of Post-hoc Newman-Keuls test, it can be seen that increasing the concentration of cadmium or aluminium in the soil does not cause a statistically significant change ($p \leq 0.01$) in content of total carbohydrates in basil extracts. Exception was the treatment with 50 mg of $AlCl_3$ per kg of dry soil and content of sugar was 31.191 mg/gDW (Fig. 4).

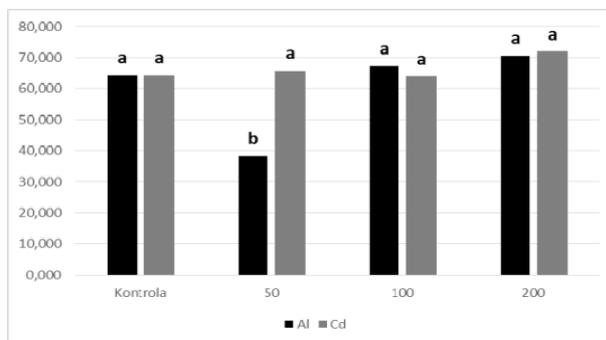


Figure 4. Variation of total carbohydrate content in basil extracts after application of aluminium and cadmium

Treatments not sharing the same letter above the column differ significantly after Newman-Keuls post-hoc ANOVA test at $p < 0.01$

Discussion

Heavy metals represent a continuous risk to all living organisms including plants. Particularly, plants have their own defence mechanism to continue survival under unfavourable conditions. In this study, *Ocimum basilicum* plants growing under the different concentrations of heavy metal (Al and Cd) was examined for their capacity to antioxidative responses to heavy metal stress. Antioxidants can prevent the accumulation of ROS. Therefore, the presence of excess heavy metals could cause oxidative stress in plants, and then increase antioxidative response during high production of ROS (Dwivedi *et al.*, 2013). Kapoor *et al.* (2014) reported positive correlation between concentration of Cd and antioxidative capacity in *Brassica juncea*. In this study both metals had positive correlation between antioxidant capacity and IC_{50} respectively.

Many studies reported production of phenolics in plants during stress conditions. Flavonoids, together with some other phenolics, contribute to the antioxidant activities of plants primarily thanks to their redox properties (Manan *et al.*, 2015). In this study Al and Cd application increased content of all examined secondary metabolites suggesting that flavonoids and other phenolics have an important role in the survival of plants under stress conditions. Therefore, after phenols content increasing, plants have ability of responding to toxicity caused by heavy metals, by removing reactive oxygen compounds and improving the metabolism of plants.

In stress conditions, plants accumulate compatible osmolytes such as proline, glycine, betaine, sugar alcohols. These compatible substances do not interfere with normal biochemical reactions and they act as osmoprotectants in plants under the stress. They provide protection to plants by preserving the integrity of proteins and membranes by rapid rehydration. During membrane and metabolism

dysfunctions, they occur in the transition between the state of decreased hydration to complete dehydration. Proline is one of the most distributed osmolyte and it helps detoxify free radicals (Niknam and Ebrahimzadeh, 2002). In our investigation, an increase of proline concentration in plants treated with these two metals was established, except for 50 mg of CdCl₂. Khavari-Nejad *et al.*, (2013) confirmed that the increase in content of proline in the extracts of different varieties of basil treated by cadmium is significant, not only because proline protects subcellular structures and macromolecules, but because it is able to protect the integrity of the protein and to increase the activity of different enzymes. Many researches established that proline stabilizes or protects natural structure of the protein regulating its hydration. In fact, the presence of water is essential to the stability of the protein structure. Therefore, proline can help maintaining the integrity and protein conformation. In general, with increasing intensity of the stress, accumulation of proline and free radicals appear in the cells. This correlation determines that the accumulation of proline is related to non-enzymatic antioxidant system. It is assumed that proline protects chloroplasts from damages (Sharmila and Saradha, 2002).

The accumulation of carbohydrates in different parts of the plant has been improved in response to different environmental stresses. The content of carbohydrates in the plant is reduced by exposure and concentration of heavy metals. Metabolism of these compounds can be affected by temperature, salinity and the presence of various ions. Increased accumulation of sugar reduces osmotic potential of the cells and another possible role of sugar is to be easily available energy source (Mohan and Hosetti, 2006; Al-Jebory, 2012).

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