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The Sorghum Plants Utilization For Accumulation of Heavy Metals

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Abstract. For phytoremediation purpose it is essential to select an appropriate plant species which should be metal tolerant with high biomass production and known agronomic techniques. The aim of this work was to expand knowledge about protection mechanisms of sorghum plants under Cd and Zn stress. The metals accumulations in roots and shoots of hydroponically grown *S. bicolor* plants were studied. Our results showed that cadmium and zinc were accumulated primarily in the roots of sorghum plants. However, higher concentrations of the metals in the solution increased their transfer to the shoots. Toxic effects of metals in the shoots, especially at lower concentrations, were not so serious compare with their influence on the roots. When GSH was added, it significantly increased the accumulation of cadmium in the roots and also in the shoots (for the highest cadmium concentration).

Keywords: heavy metals, Sorghum, toxicity, accumulation

1. Introduction

Heavy metal pollution of soil is usually related to human activities. Sites near mining activities or heavy industry are often highly contaminated with toxic metals. Besides, application of fertilizers increased i.e. cadmium content in topsoils (Lado et al., 2008). Such polluted soil is hardly usable for agricultural purposes because the pollution can be transferred to a food chain. Heavy metals bounded in the soil are leachable and they can also spread further via ground water. To avoid the spread of contaminants it is possible to use phytoremediation techniques which can immobilize or decrease the pollution (Salt et al., 1998). Plants are able to immobilize metals in soil by forming of insoluble compounds as a result of interactions of contaminants with plant exudates in rhizosphere or by absorption on root system (Kidd et al., 2009). Some plant species are also able to accumulate heavy metals in their plant tissues so the contaminant is removed from site with harvested plant (McGrath and Zhao, 2003).

For phytoremediation purpose it is essential to select an appropriate plant species which should be metal tolerant with high biomass production and known agronomic techniques. The above mentioned conditions met woody plants, grasses, and crop plants. The primarily interest concerning biomass crops now is focused on energy crop i.e. *Miscanthus giganteus*, *Salix* sp., *Populus* sp., *Zea mays*, and *Sorghum* sp.

Sorghum bicolor is C4 grass widely used as a forage crop. It is the fifth most important cereal in the world. In addition, sorghum plants are multipurpose cereals of potential interest for several non-food uses, especially as energy crops (Barbanti et al., 2006). The crop is resistant to drought, temperature, and toxic pollution. It was shown that sorghum plants were able to accumulate large quantities of Cd, Cu, Pb, and Zn in shoots and their biomass production was higher than sunflower or corn biomass production (Epelde et al., 2009; Zhuang et al., 2009). Moreover, other studies concluded that sorghum plants were highly tolerant to metal pollution and capable of reaching high biomass values in the presence of metals (Pinto et al., 2004; Hernández-Allica Jakubet al., 2008).

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The aim of this work was to expand knowledge about protection mechanisms of sorghum plants under Cd, Cu and Zn stress. The metals accumulations in roots and shoots of hydroponically grown *S. bicolor* plants were studied.

2. Material and Methods

2.1. Plant material

Sorghum bicolor L. plants (cultivars Honey Graze, Expres, DSM 14-535, Nutri Honey, Sweet Virginia and Sucrosorgo 506) (from SEED SERVICE s.r.o., Czech Republic) were cultivated in a cultivation room under controlled conditions (23°C, humidity about 60%, and daily light phase of 16 hours) in modified Hoagland's solution (Hoagland, 1920) at pH 5.5. Four weeks old plants were used for experiments.

2.2. Experiment designs

Firstly, sorghum seedlings cv. "Nutri Honey" were cultivated in Erlenmeyer flasks (each plant per one flask) in 300 mL of modified Hoagland's liquid solution as a control or in a solution with cadmium in three concentrations (100, 200 or 500 μM) or with zinc in four concentrations (200, 500, 1000 or 5000 μM). Samples were taken at 0, 14, 21 and 28 days.

Secondly, sorghum seedlings of six cultivars (Honey Graze, Expres, DSM 14-535, Nutri Honey, Sweet Virginia and Sucrosorgo 506) (Seed Servis, Ltd., Czech Republic) were cultivated in Erlenmeyer flasks (each plant per one flask) in 300 mL of modified Hoagland's liquid solution as a control or in a solution with cadmium in two concentrations (100 or 200 μM) or with zinc in two concentrations (2000 or 5000 μM). Samples were harvest after 28 days of cultivation.

Finally, sorghum seedlings cv. "Nutri Honey" were cultivated in Erlenmeyer flasks (each plant per one flask) in 300 mL of modified Hoagland's liquid solution as a control or supplemented by EDTA (Penta, Czech Republic) or glutathione (Sigma – Aldrich) at concentration 100 μM. The solutions were supplied with cadmium in three concentrations (50, 100 or 500 μM) or with zinc in four concentrations (100, 500 or 1000 μM). Samples were taken at the beginning and after 7 days.

2.3. Sample processing and metal determination

The roots of plants were washed subsequently by double distilled water, solution of EDTA (concentration 0.1 M), and again double distilled water. The plant samples were separated into two parts (leaves and roots) and then they were weighted. All samples were frozen at –80 0C in liquid nitrogen and stored in a freezer. Lyophilized plant samples were used for heavy metal determination. In all experiments there were four replications for each treatment and each concentration. For heavy metals treatments $Cd(NO_3)_2$ (Penta, Czech Republic) and $Zn(NO_3)_2$ (Lach-Ner, Ltd., Czech Republic) compounds were used.

The dried plant tissues were ground to a powder, and digested in 5 ml of acid mixture of $HClO_4$ and HNO_3. Content of Cd and Zn was measured by atomic absorption spectroscopy SensAA (GBS, Australia).

3. Results and Discussion

In the time dependence experiment the cadmium concentration in roots was generally greater than in shoots. More than 10 times higher cadmium content (Fig.1) and 2 times higher zinc content (Fig. 2) was present in roots. The roots seem to have a barrier to prevent the transport of cadmium to shoots, but for zinc this barrier is more permeable. Even so that cadmium is an acompanion element of zinc and has very similar properties as zinc. A higher metal uptake in roots relative to shoots is reported in grasses, semi-resistant, sensitive and resistant plants including sorghum (Pinto et al. 2004; Abo-Kassem et al., 1997; Obata and Umebayashi, 1997; Ouariti et al., 1997).

Figures show the accumulation of cadmium and zinc in roots and shoots (Fig. 3 and 4). There was significant difference in cadmium concentrations accumulated in the Honey Graze, DSM 14-535, Nutri Honey, Sweet Virginia, Expres and Sucrosorgho 506 cultivars. The highest cadmium concentrations in roots ranged from 0.44 to 1.08 mg/kg, which were nearly 10-fold higher than the concentrations (approximately 0.14 mg/kg) in shoots. The cadmium concentrations in roots and shoots of sorghum plant grown in control medium varied between 0.003 and 0.013 mg/kg, with the highest concentration in roots of Nutri Honey and the lowest in roots of Sucrosorgho 506.

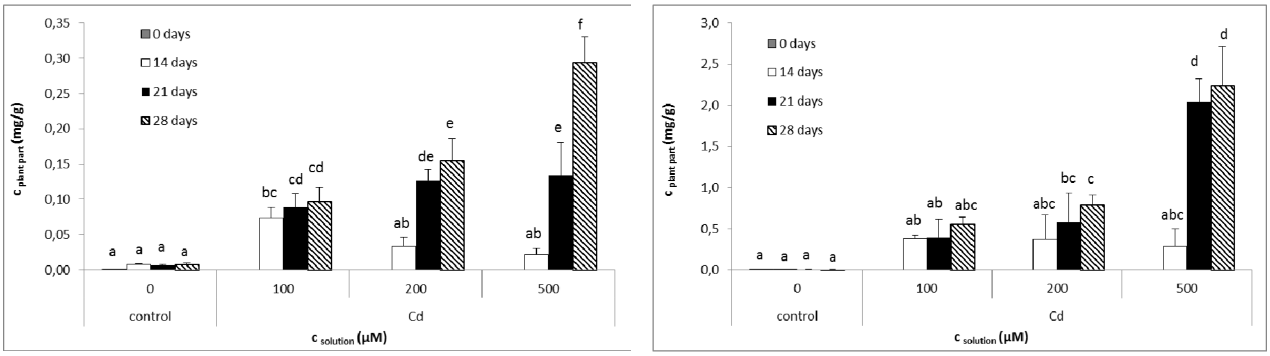

Fig. 1: Cadmium concentrations [mg/g] in shoots and roots of *S. bicolor* during 28 days of growth in solution with 0, 100, 200 or 500 [µM] concentrations.

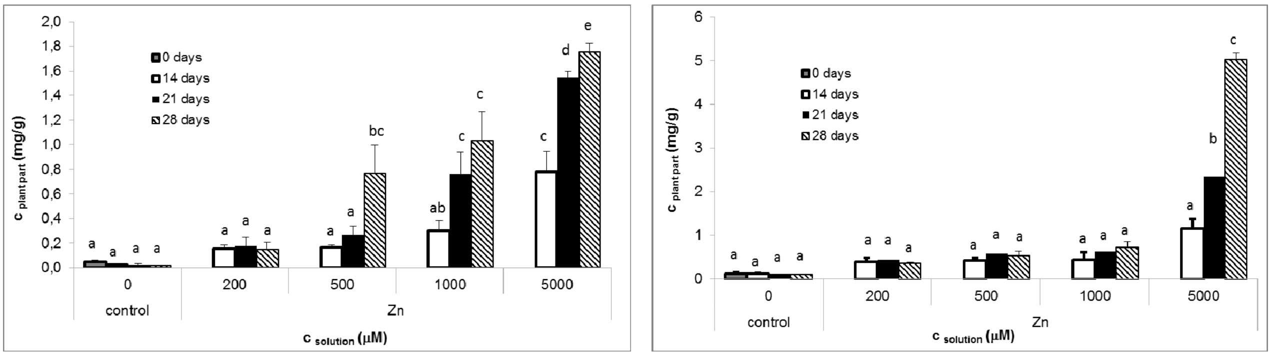

Fig. 2: Zinc concentrations [mg/g] in shoot and roots of *S. bicolor* during 28 days of growth in solution with 0, 200, 500, 1000 or 5000 [µM] concentrations.

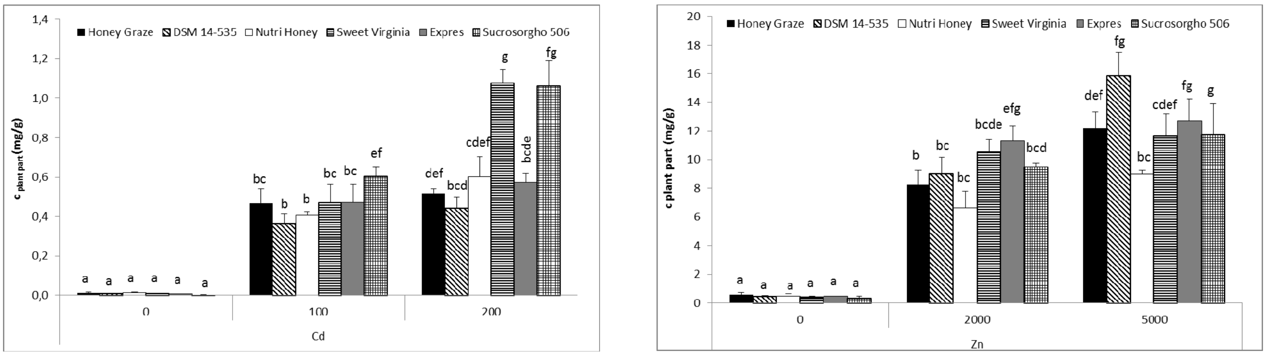

Fig. 3: Cadmium or zinc concentrations [mg/g] in root of six cultivars of *S. bicolor* after 28 days of growth in solution with 0, 200 or 500 [µM] cadmium concentrations or with 0, 2000 or 5000 [µM] zinc concentrations.

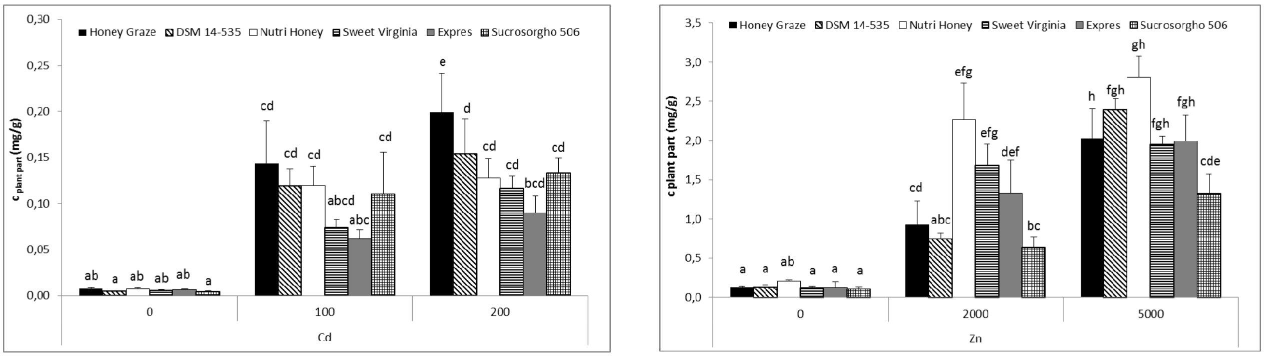

Fig. 4: Cadmium or zinc concentrations [mg/g] in shoot of six cultivars of *S. bicolor* after 28 days of growth in solution with 0, 200 or 500 [µM] cadmium concentrations or with 0, 2000 or 5000 [µM] zinc concentrations.

The highest zinc concentrations in roots of DSM 14-535, Expres and Honey Graze were 15.85, 12.68 and 12.20 mg/kg, respectively. Zinc accumulation in shoots of Nutri Honey reached 2.80 mg/kg with 5 mM zinc application. The rate of cadmium transport to shoot in DSM 14-535 and Honey Graze was found to be higher than in others (Nutri Honey was found as third the best). For the zinc the best rate transport for Nutri Honey was found. It has been reported that different cultivars of sorghum have a different ability to accumulate heavy metals from contaminated soils (Zhuang et al., 2009). These findings are consistent with our results.

The goal of addition of the chelating agent EDTA or glutathione (GSH) was to increase the ability of sorghum accumulate zinc and cadmium. Addition of GSH significantly increased the accumulation of cadmium in the roots at all tested concentrations and also in shoots at the highest cadmium concentration (0.5 mM) (Fig. 5). In sorghum, glutathione-mediated cadmium uptake was higher than free cadmium uptake. A similar increase of uptake was also found for example in *Pteris vittata* accumulating arsenic (Wei et al., 2010). Increase of cadmium uptake may be caused by Cd-GSH complex, which is transferred to the roots using some peptide transporters of PTR family (Vadas and Ahner, 2009). These transporters are also having been shown to transport such GSH conjugates (Zhang et al., 2004) and metal complexes with GSH (Cagnac et al., 2004). At least one of the transporters is even transcriptionally regulated by cadmium (Bogs et al., 2003). Another reason may be that the GSH is precursor of synthesis of phytochelatins, which are able to chelate cadmium and zinc, and whose synthesis is induced mainly just cadmium (Cobbett and Goldsbrough, 2002).

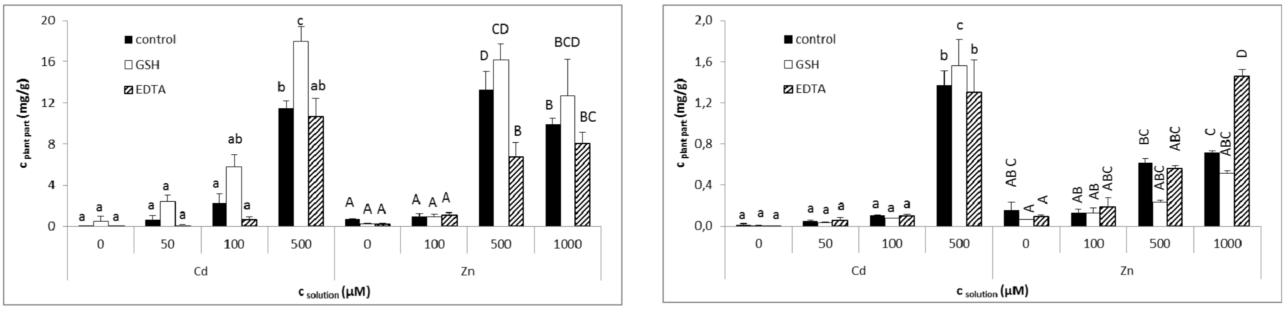

Fig. 5: Cadmium and zinc concentrations [mg/g] in root and shoots of *S. bicolor* after 7 days of growth in solution with concentrations 0, 50, 100 or 500 [µM] of cadmium or with concentrations 0, 100, 500 or 1000 [µM] of zinc. Solution was supplemented by GSH or EDTA.

Increase of zinc uptake after the addition of GSH was observed only in the roots, but in comparison with the control, the increase was not significant. In the shoots the zinc uptake were even reduced compared to the control. Similar results were obtained Vadas and Ahner (2009) with *Zea mays* and *Brassica napus* cultivated with lead. Addition of EDTA resulted in a reduction of uptake of cadmium in both roots and shoot as compared to control (Fig. 5). Xu et al., (2007) published reduction of the uptake of lead by sorghum plants in both the roots and shoots at low concentration of EDTA (ratio Pb/EDTA was from 1:0 to 1:2). When the Pb/EDTA ratio was 1:4, an increase to twice in lead accumulation at the ratio of 1:1 was found, but it was significantly lower than accumulation in the absence of EDTA. Reduced uptake of cadmium with increasing concentration of EDTA was also observed in *Juncus effusus* (Najeeb et al., 2011). Positive effect on zinc uptake after addition of EDTA was found only in shoots of barley, potato, Indian mustard, and white lupin. In roots we found reduced zinc uptake compared to the control. Increasing concentrations of EDTA resulted in higher zinc accumulation in shoots as described Collins et al. (2002).

4. Conclusion

Tested metals (Cd and Zn) were accumulated primarily in roots. However, higher concentrations of the metals in the solution increased their transfer to the shoots. The shoot translocation may be the avoidance of toxic effect of the metal accumulation in the roots. Toxic effects on shoots, especially at lower concentrations, were not devastated compare influence on roots.

5. Acknowledgements

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