2012 3rd International Conference on Biology, Environment and Chemistry
IPCBEE vol.46 (2012) © (2012) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2012. V46. 6

Phytostabilization or Accumulation of Heavy Metals by Using of Energy Crop Sorghum sp.

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Abstract. Phytostabilization is a method that exploits plants to immobilize contaminants in soil or ground water using roots absorption, adsorption onto the surface of the roots, or formation of insoluble compounds as a result of interactions of contaminants with plant exudates in rhizosphere. This method reduces the mobility of contaminants and thus prevents their migration into the groundwater or into the air. Therefore, phytostabilization can be used for restoration of the vegetative cover at sites where the original vegetation disappeared due to a high content of metals in the soil. Plant species that are tolerant to high concentrations of metals can reduce migration of contaminants by wind erosion of exposed soil surfaces and at the same time they can reduce leaching of contaminants into the groundwater.

The project is focused on increasing the production of energy crops (*Sorghum* sp.)and also to immobilize heavy metals in soils contaminated by industrial activities. The knowledge about energy crops cultivation on contaminated soils should provide new possibilities for solving problems with contamination of water and soil, which we are facing around the world.

Keywords: Sorghum, Heavy metals, Phytostabilization, Accumulation

1. Introduction

Sorghum bicolor L. is an important crop due to its wide use as food, feed and energy crop. In addition, sorghum appears promising as a cereal crop, which has some non-food uses, particularly for bioethanol production [1]. It comes originally from North Africa, and therefore is resistant to heat and drought. It is unique due to a specific compounds contents (phytates, tannins), which affect its nutritional value. Increased concentrations of essential elements in agricultural soils mean higher plant uptake. However, soil also contains toxic elements, such as heavy metals, and the higher concentration, the higher uptake of nonessential elements is. What’s more, the concentrations of these metals are so toxic they can cause death of plants or reduce their production.

Sorghum is able to accumulate large quantities of metals in shoots grown in hydroponic conditions but in field trials these amounts are lower [1]. Nevertheless, one advantage is the possibility of some economic returns during the process. *Sorghum* has high production of biomass in comparison with other crops such as sunflower or corn [2]. Nowadays, there is a huge energy demand; therefore, the growth of energy crops on polluted sites can be feasible.

In this study, we dealt with metals accumulation in roots and shoots of hydroponically growing *S. bicolor* plants. We also focused on the temporal sequence of physiological reactions, including changes in chlorophylls contents and antioxidative enzymes activities.

2. Material and Methods

2.1. Plant Material

Sorghum bicolor L. plants were cultivated in a cultivation room under controlled conditions (23°C, humidity about 60%, and daily light phase of 16 hours) in Hoagland’s solution [3]. Four weeks old plants were replaced into solution with 50, 100, 200, 500, 1000, 2000 or 5000 μM concentration of Cd or Zn ions. Plants were harvested after 7 days, divided to shoots and roots, and freeze-dried.

2.2. Metal Determination

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).

2.3. Pigment Analysis

Pure methanol (10 ml) was added to approximately 0.5 g freshly ground leaf material. The mixture was stored in the dark at 4°C. The supernatant was collected in Falcon tubes next day. Extracted pigments were determined spectrophotometrically at the following wavelengths: 665.2, 652.4 and 470 nm [4]. Chlorophyll (Chl) a/b ratio was calculated from Chl a, and Chl b measured as mg/g of fresh weigh of the leaf.

2.4. Enzyme Assays

All enzyme assays were performed in a microplate reader TECAN Infinite N200. Peroxidase (POX) activity was detected on the base of colour reaction with guaiacol [5]. Glutathion-S-transferase (GST) activity was detected on the base of reaction with CDNB [5]. The enzyme activity was expressed in μkat/mg protein. The protein extraction followed a procedure by Bradford using serum albumin as standard [6].

2.5. Root Measurement

Roots of each plant were washed from Perlit and measurement was done on scanner EPSON PERFECTION V700 PHOTO. The results were processed by software WinRHIZO.

2.6. Leaves Counting

Leaves of each plant were counted when the experiment was start and at sampling point (14, 21 and 28 days of experiment). The results were processed as a ratio of number of leaves at sampling day and number of leaves at start of experiment.

3. Results and Discussion

Our results showed that metals accumulation in plants increased with its increasing concentration in the growing solution; although, the metals distribution in plant parts was various (Fig. 1, 2). Tested metals (Cd and Zn) were accumulated primarily in roots. However, higher concentrations of the metals in the solution caused an increase transfer of the metals to the shoots. Metals absorption and the restriction of translocation to the shoots may be the avoidance of toxic effect of the metal on the roots.

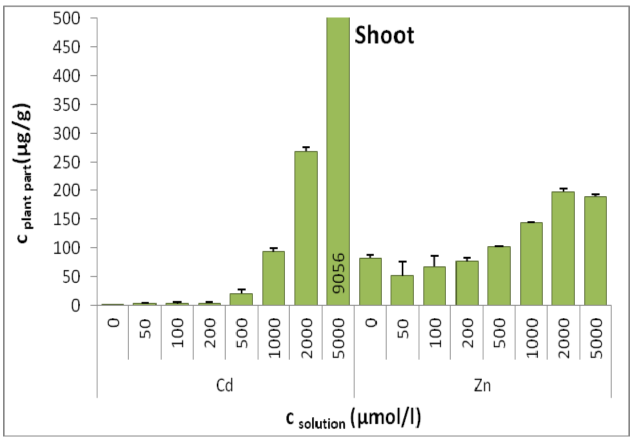

Fig. 1: Cadmium and zinc concentrations [μg/g] in shoot of *S. bicolor* after 7 days of growth in solution with 0, 50, 100, 200, 500, 1000, 2000 or 5000 [μmol/l] concentration; standard deviation is represented as ± S.D. (n = 3).

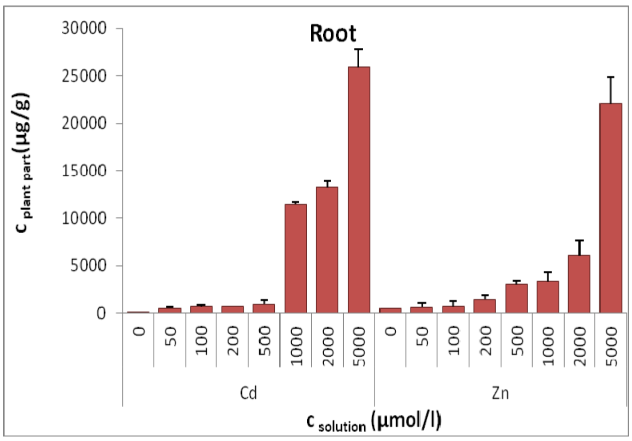

Fig. 2: Cadmium and zinc concentrations [μg/g] in root of *S. bicolor* after 7 days of growth in solution with 0, 50, 100, 200, 500, 1000, 2000 or 5000 [μmol/l] concentration; standard deviation is represented as ± S.D. (n = 3).

The toxic effect of tested metals in the leaves was also obvious. The increase in the Chl a/b ratio indicated a greater depletion of the Chl b pool (Fig.3). Chl b was converted to Chl a, thus Chl a/b ratio increased. It was reported that the increase of Chl a/b ratio followed the chlorosis progression [7]. These observations suggested a specific pattern of Chl loss during metal-induced chlorosis, representing either a direct effect of metals on the two Chl pools or an indirect effect created by the metals.

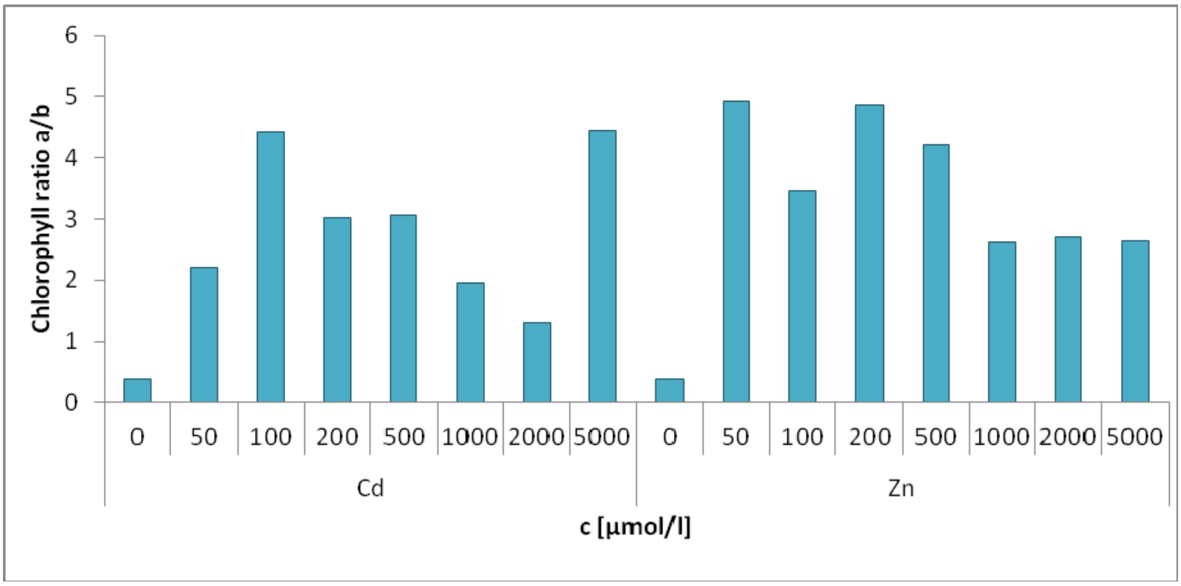

Fig. 3: Chl a/b ratio in the leaves of *S. bicolor* after 7 days of growth in solution with 0, 50, 100, 200, 500, 1000, 2000, or 5000 [µmol/l] concentration.

In the root, POX activity was decreased; whereas, in the shoot the activity was comparable to control (Fig.4). The activity in the shoot corresponded with low metal accumulation. When cadmium level increased, POX activity also rised (at 1000 µmol/l). At higher concentration levels the free radicale mission in the root was beyond the quenching capacity of POX which in turn might contribute to reduced enzyme activity. It was reported that the main response to heavy metals is an increase in POX activities as long as the stress is not too strong for the plant's defense capacity [8].

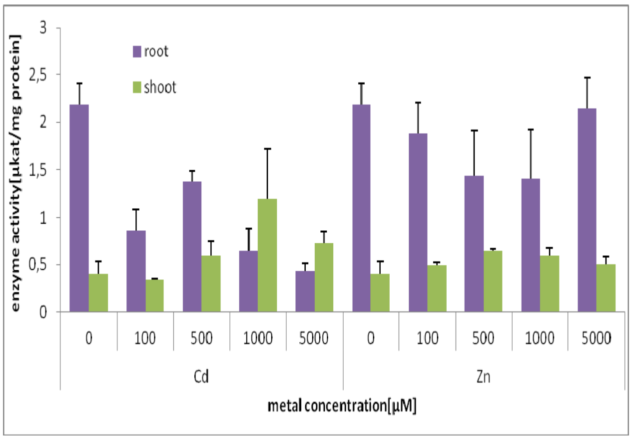

Fig. 4: Peroxidase activity [µkat/mg protein] in root and shoot of *S. bicolor* after 7 days of growth in solution with 0, 100, 500, 1000 or 5000 [µmol/l] concentration; standard deviation is represented as ± S.D. (n = 6).

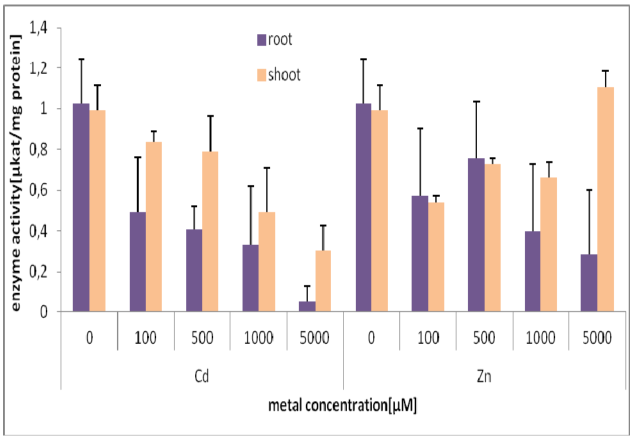

Fig. 5: Glutathion-S-transferase activity [µkat/mg protein] in root and shoot of *S. bicolor* after 7 days of growth in solution with 0, 100, 500, 1000 or 5000 [µmol/l] concentration; standard deviation is represented as ± S.D. (n = 6).

In the root and shoot affected by cadmium, GST activity was decreased; whereas, in the root affected by zinc was decreased and in the shoot the activity was increased (Fig.5). The activity in the shoot affected by zinc corresponded with rised metal accumulation.

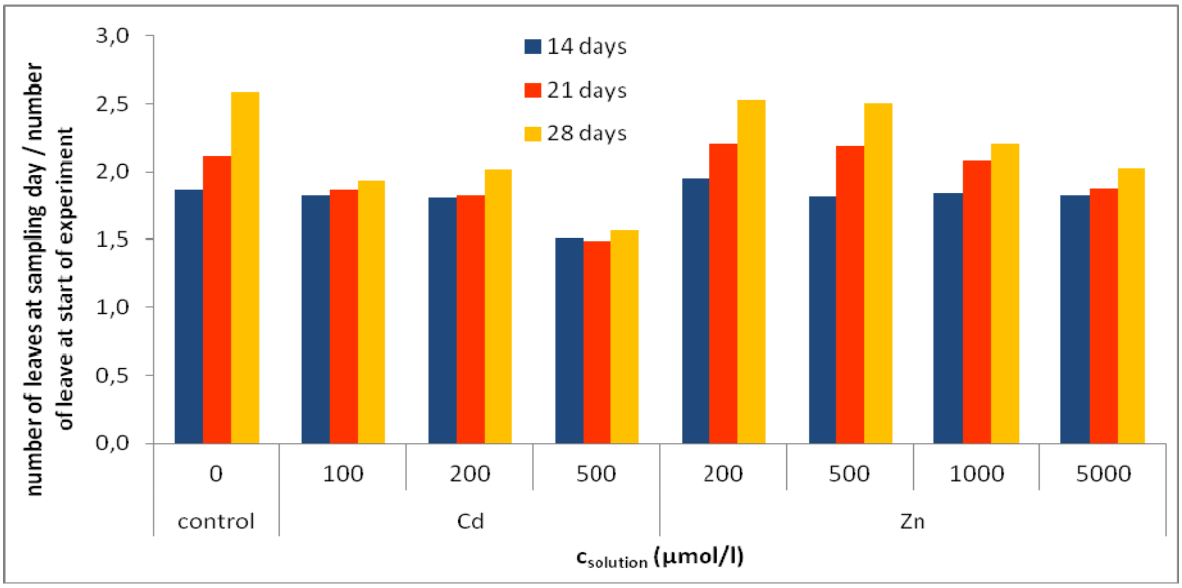

Fig. 6: Number of leaves at sampling day and number of leaves at start of experiment ratio of *S. bicolor* after 14, 21 and 28 days of growth in solution with 100, 200 or 500 [µmol/l] cadmium concentration or with 200, 500, 1000 or 5000 [µmol/l] zinc concentration; standard deviation is represented as ± S.D. (n = 4).

Number of leaves was decreased with higher metal concentration. Cadmium was more toxic than zinc. The reduction of leaves was visible only at highest zinc concentrations (1000 and 5000 µmol/l) (Fig. 6). The highest concentrations of both tested metals resulted in yellowish or brownish leaves and in majority in dry shoots.

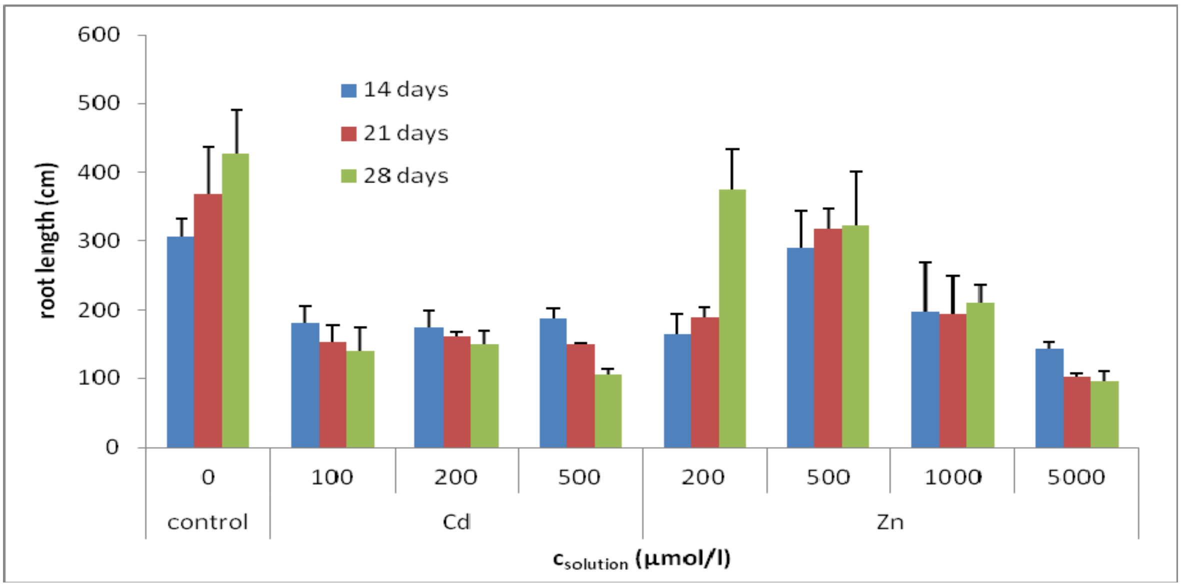

Fig. 7: Root length [cm] of *S. bicolor* after 14, 21 and 28 days of growth in solution with 100, 200 or 500 [µmol/l] cadmium concentration or with 200, 500, 1000 or 5000 [µmol/l] zinc concentration; standard deviation is represented as ± S.D. (n = 4).

Roots length decreased with higher metal concentration except low zinc concentration where root induction was observed (Fig. 7). Cadmium was more toxic than zinc. The low cadmium concentration shows significant root length reduction (half length compare to control roots).

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. On the other hand, increased transfer of the metals into the leaves caused the toxic effect that led to an increase of Chl a/b ratio. Moreover, higher concentration levels in the roots were beyond the quenching capacity of POX and GST so enzymes activity was reduced. Toxic effects on shoots, especially at lower concentrations, were not devastated compare influence on roots.

5. Acknowledgements

This work was supported by project Kontakt No. LH 12162 (Ministry of Education, Youth and Sports).

6. References

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