

## BIOACCUMULATION OF CADMIUM BY SPRING BARLEY (*HORDEUM VULGARE* L.) AND ITS EFFECT ON SELECTED PHYSIOLOGICAL AND MORPHOLOGICAL PARAMETERS

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### ABSTRACT

Heavy metals and other toxic elements in the environment, mainly located in soil and groundwater, have a significant effect on plant and its productivity that has a huge attention in recent years. Accumulation of heavy metals in soil cause toxicity to plants, and contaminate the food chain. The industrial areas, as well as developing countries have been contaminated with high concentration of heavy metals. Main sources of contamination are mining and other industrial processes, as well as military and or landfills, sludge dumps or waste disposal sites. The heavy metals are very dangerous to environment and pose serious danger to public health by entering through the food chain or into drinking water. Phytoextraction is one way how to remove the contaminants from soil by plants. Phytoextraction of heavy metals is a technology that has been studied for several years. It is more ecological and cheaper way how to clean our environment. Several plant species are known because they hyperaccumulate a high contents of metals from the soil. The accumulators are mainly herbaceous species, crops and nowadays angiosperm trees with a high growth such as poplars or willows. We have focused on the determination of some morphological (length and weight of roots and biomass) and physiological (contents of dry mass and number of leaf stomata) characteristics and the determination of the bioaccumulation factor and the translocation factor of cadmium by spring barley (*Hordeum vulgare* L.). Imprints of leaves were evaluated using an optical microscope Axiostar Plus, Carl Zeiss, lens CP Achromat 40x/0.65, eyepiece PI 10x / 18, Canon Utilities Software Zoom Browser EX 4.6 and hardware Acer Travel Mate 4600, Canon Power Shot A95. The density of stomata was evaluated on an area of 1 mm<sup>2</sup>. Samples of the dried plants (leaves and roots) were mineralized by acid digestion using microwave digestion device MARS X - press 5. The end of determination to obtain the cadmium content was performed by atomic absorption spectrometer Varian 240 Z with GTA120 graphite furnace. The effect of contamination by cadmium to germination, length of leaves and number of stomata on abaxial side of leaf was confirmed. The contaminated soil by cadmium does not pose a risk of heavy metal entry into the feed and food chain by spring barley (*Hordeum vulgare* L.).

**Keywords:** stomata; barley; phytoextraction; cadmium; heavy metals;

### INTRODUCTION

Trace metals in the aquatic environment can be traced to both natural and anthropogenic sources. Trace metals are classified as being light or heavy with densities less or greater than 5 g.cm<sup>-3</sup>. Natural and anthropogenic activities usually result in gaseous emissions and wastewater discharges into the environment. When these substances in the emissions and effluent discharged into the environment are in very minute amounts or in low concentrations and are toxic to plants and animals and have short residence time in the environment, they are described as contaminants (Tyokumbur and Okorie, 2014).

Heavy metals are extremely persistent in the environment because they are not biodegradable and may not be broken down by chemical oxidation or through thermal processes. Some metals are essential for plant growth. Very high or low contents of some heavy metals may be inhibitory to plant growth (Ochonogor and Atagana, 2014).

Heavy metals are inorganic chemical hazards. The most contaminated sites are by lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni). Soils are the major sink for heavy metals released into the environment by. Their total concentration in soils persists for a long time after their introduction. Changes in their chemical forms (speciation) and bioavailability are possible (Maslin and Maier, 2000).

Heavy metal contamination of soil may pose risk human's health and the ecosystem through soil, the food chain, drinking ground water, reduction in food quality (safety and marketability) via phytotoxicity, reduction in land usability for agricultural production causing food insecurity, and land tenure problems. The adequate protection and restoration of soil ecosystems contaminated by heavy metals require their characterization and remediation (Wuana and Okieimen, 2011).

Cadmium (Cd<sup>2+</sup>) is a highly toxic trace element whose presence in the environment is caused by human

activities. It is taken up by roots via essential metal transporters (Cohen et al., 1998; Lasat et al., 2000; Pence et al., 2000).

After longer exposure to heavy metal decreases growth rate by affecting various aspects of plant physiology, as well as decreases carbon assimilation that can lead to wilting (Perfus - Barbeoch et al., 2002).

Plants throughout their life cycle experience various types of environmental stresses (such as drought, salinity, high temperature, cold, heavy metal and other similar stresses) due to their sessile nature. Among these stresses, salinity stress has become the limiting factor for the productivity of agricultural crops by affecting germination, plant vigor and finally crop yield (Munns and Tester, 2008; Zhang et al., 2011; Arif Shafi Wani, 2013).

Cadmium is toxic to many plant species even at very low concentration. It is mainly generated from smelting industries, abrasion of automobile tires, burning of diesel and heating oils and from phosphate fertilizers originated by aerobically digested sewage sludge. Concerning to its effects on plants, Cd is accumulated in them and interacts with several physiological processes such as photosynthetic, respiratory and nitrogen metabolism, resulting in poor growth and low biomass. Furthermore, Cd is associated with oxidative stress and it can result in the production of free radicals and active oxygen species (Puertas-Mejía et al., 2010).

The role of oxidative stress in metal toxicity has been assessed by measuring alterations in the redox metabolic components of stressed plants. Over the past few years major progress has been achieved, particularly by comparing metal tolerant and/or metal hyperaccumulator genotypes with their non-tolerant relatives and by using transgenic plants that overexpress or lack specific redox elements. These approaches provided novel insight into the relationship between metal sensitivity and cellular redox imbalance (Sharma, 2008). Metal ions may directly interfere with the metabolic activities by altering the conformation of proteins, for example enzymes, transporters or regulator proteins, owing to their strong affinities as ligands to sulfhydryl and carboxylic groups. This is taken to be a major cause for metal imposed toxic effects (Sharma, 2004). Stress factors generally applied at higher levels may cause irreversible changes in physiological processes as stomatal closure or slowing down the biochemical processes. Low levels of toxic metals such as cadmium also slow growth and affect biochemical processes. Strength and duration of stress exposure can also cause permanent changes. In addition to toxic metals, changes in the membranes of plant cells are mostly affected by water stress, changes in temperature and by frost. Toxicity of the metals (such as cadmium), can cause an accumulation in tissues, which consequently affects the metabolism of plants, particularly the photosynthetic apparatus (Lachman et al., 2015).

The mechanisms of cadmium (Cd) uptake and tolerance in plants have been studied extensively, but a clear understanding of what controls the translocation of Cd to aboveground tissues is lacking. One approach to better understanding the factors that control Cd accumulation and distribution is to determine where Cd is bound as it

travels from the root surface to aboveground parts (Akhter et al., 2014).

Accumulation and translocation of the environmental pollutants as cadmium was evaluated in different parts of plants. Although roots comprise usually only a little part of whole plant biomass, they consistently contain 70 – 100% of the whole plant metal burden (Lachman et al., 2004).

The effect of Cd on transpiration of water from leaves has been studied extensively. At low concentrations, Cd increased the permeability of the leaf cuticle and increased transpiration in sugar beet. At high concentrations, Cd induced stomatal closure and decreased leaf transpiration in mustard (*Brassica juncea* L.), barley (*Hordeum vulgare* L.), and lettuce (*Lactuca sativa* L.). However, the mechanism of Cd-induced stomatal closure is still poorly understood. Some studies reported increased production of abscisic acid (ABA) with increased Cd-exposure and suggested that ABA might regulate stomata closure in Cd-stressed conditions, however, in ABA-insensitive mutants of *Arabidopsis thaliana* L. Cd<sup>2+</sup> affected guard cell regulation in an ABA-independent manner by entering the cytosol via Ca<sup>2+</sup> channels (Akhter and Macfie, 2012).

Environmental contamination by Cd in human food typically comes from crops and contaminated water. The effects of Cd range from shortness of breath, effects on respiratory system, vomiting and diarrhoea, kidney damage and renal failure, bone damage, Itai-Itai disease (osteomalacia), to low birth weight and increase in abortions (Stanbrough et al., 2013).

Crops grown in contaminated soil may accumulate Cd in different plant parts, such as root, leaf, grain etc., and consumers may develop a number of Cd-related chronic diseases. It is recommended to keep Cd concentrations below regulatory guidelines in vegetables, fruits, grains and other agricultural products to avoid metal toxicity. Because the concentration of Cd in edible plant tissues is not always directly proportional to the concentration of Cd in the soil, understanding the mechanisms of Cd accumulation and translocation in plants is important to ensuring food safety (Akhter and Macfie, 2012).

On the other hand cereals are main foods in many countries, as human foods or as animal feeds. Epidemiological studies indicate that the consumption of whole - grain and whole - grain products is related to reduction in total mortality, coronary heart disease mortality, diabetes and cancer incidence. These beneficial effects are attributed to the bioactive factors in cereal grain such as non digestible carbohydrates and phytochemicals (Ivanišová et al., 2010). Cereals and pseudocereals have a significant role in human nutrition. They are source of specific carbohydrates, proteins, lipids, fibre and wide spectrum of vitamins and minerals. Cereals and pseudocereals may also contain some antinutritional factors, such as phytic acid, polyphenols, trypsin inhibitors and inhibitors of  $\alpha$ -amylase. These are responsible for reducing of protein and carbohydrate digestibility and decreasing accessibility of minerals due to complex formation (Kocková and Valík, 2011).

The legislation should respect environmental protection and public health, at national and international level (Kabata - Pendias and Pendias, 2001).

**MATERIAL AND METHODOLOGY**

The aim of our work is the evaluation of selected morphological (length and weight of roots and biomass) and physiological (contents of dry mass and number of leaf stomata) characteristics and the determination of the bioaccumulation factor and the translocation factor of cadmium by spring barley (*Hordeum vulgare* L.).

The seeds were germinated in Petri dishes on a filter paper for 48 hours in the dark with temperature 25 °C and 80% air humidity. After 2 days 100 germinated seeds were transferred into each container filled with 950 g of washed silica sand. The containers were watered firstly by Hoagland's solution (Hoagland and Arnon, 1950) and after that on alternate days as needed with distilled water. The water – soluble CdCl<sub>2</sub> · 2.5 H<sub>2</sub>O was added to containers to obtain the application of 1, 5 and 25 mg.kg<sup>-1</sup>. The control treatments (0 mg.kg<sup>-1</sup>) had no added heavy metal. The plants were grown with supplementary lighting 16/8 hours photoperiod and controlled temperature 20 – 25 °C. The plants were harvested after four weeks of cultivation. They were cleaned, washed with deionized water and separated into roots and aerial parts.

Imprints of leaves have been transferred to a glass slide and preparations were made for further analysis. Microreliefs we collected in the central part of the leaf on adaxial (upper) and abaxial (lower) side. Preparations were evaluated using an optical microscope Axiostar Plus, Carl Zeiss, lens CP Achromat 40x/0.65, eyepiece PI 10x / 18, Canon Utilities Software Zoom Browser EX 4.6 and hardware Acer Travel Mate 4600, Canon Power Shot A95. The density of stomata was evaluated on an area of 1 mm<sup>2</sup>.

Cadmium concentrations were obtained by treating the samples by 10 cm<sup>3</sup> of aqua regia (2.5 cm<sup>3</sup> HNO<sub>3</sub> and 7.5 cm<sup>3</sup> HCl) using microwave digestion unit Mars Xpress 5 (CEM Corp., USA). The mineralization was carried out in teflon vessels. The concentrations were measured by atomic absorption spectrometry (AAS) in a Varian AA 240 Z (Varian, Australia) with GTA120 graphite furnace.

The significance of selected parameters was verified by

LSD test. We used Pearson correlation coefficients at significance level of  $p < 0.05$  (weak statistical significance) and  $p < 0.01$  (very strong statistical significance) by STATGRAPHICS Plus 5.1.

**RESULTS AND DISCUSSION**

In the experiment, the barley plants showed visual symptoms of the external toxic effect of metal, such as leaf discoloration and dehydration.

The changes in dry matter content of roots and leaves, and the length of the leaves indicate that the plant reacts to changing of environmental conditions (Piršelová et al., 2010).

Strong statistical significance was confirmed between contamination by cadmium with (Table 1):

- germination → low negative correlation,
- length of the leaves → high negative correlation,

Weak statistical significance was confirmed between contamination by cadmium with (Table 2):

- weight of biomass → low negative correlation.

The negative impacts of heavy metals on plants are decreasing of seed germination, lipid content, enzyme activity and plant growth, the inhibition of photosynthesis or reduction of chlorophyll production (Gardea-Torresdey et al., 2005; Akpor et al., 2014).

The adaxial (Figure 1) and abaxial (Figure 2) side preparations of the barley leaves were evaluated using an optical microscope. Very strong statistical significance was between contamination by cadmium with (Table 3) number of leaf stomata on abaxial side → high positive correlation. After microwave digestion of the harvested biomass (roots and leaves) of spring barley (*Hordeum vulgare* L.) was measured the content of Cd and the obtained results are shown in Figure 3 and Figure 4.

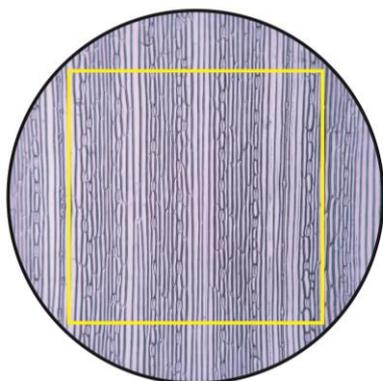
The cadmium content in roots in first variant (application 1 mg.kg<sup>-1</sup> of CdCl<sub>2</sub> · 2.5 H<sub>2</sub>O) was 219.39 ± 68.65 mg.kg<sup>-1</sup>, in second variant (application 5 mg.kg<sup>-1</sup> of CdCl<sub>2</sub> · 2.5 H<sub>2</sub>O) was 489.38 ± 140.41 mg.kg<sup>-1</sup> and in third variant (application 25 mg.kg<sup>-1</sup> of CdCl<sub>2</sub> · 2.5 H<sub>2</sub>O) was 2064.36 ± 108.32 mg.kg<sup>-1</sup> of dry mass.

**Table 1** Cadmium effect on the germination and length of the leaves of barley.

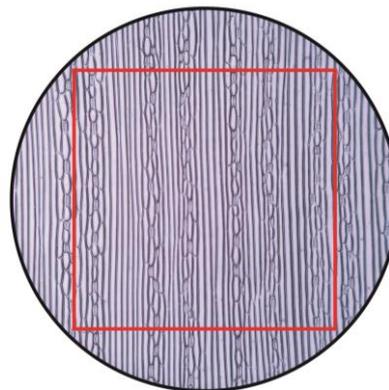
Contamination by Cd (mg.kg <sup>-1</sup> )	Germination (%)	Length of the leaves (cm)
0	94	29.40
1	91	28.60
5	88	24.90
25	84	23.90

**Table 2** Cadmium effect on the dry weight of the roots and leaves of barley.

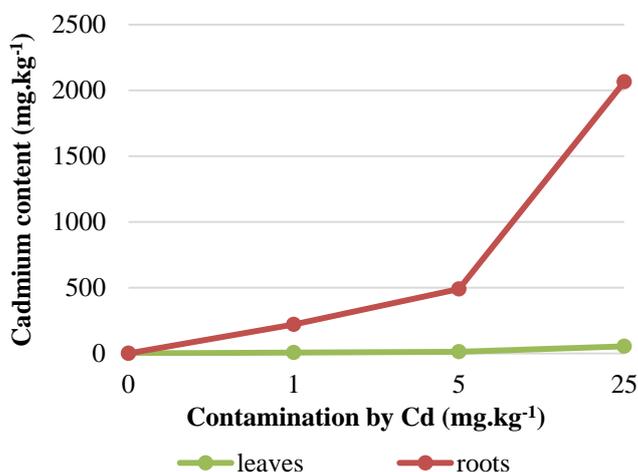
Contamination by Cd (mg.kg <sup>-1</sup> )	Dry weight of the roots (g)	Dry weight of the leaves (g)	Total biomass (g)
0	0.8902	1.4850	2.3752
1	0.7232	1.4848	2.2080
5	0.4792	1.2613	1.7405
25	0.6400	1.2850	1.9250



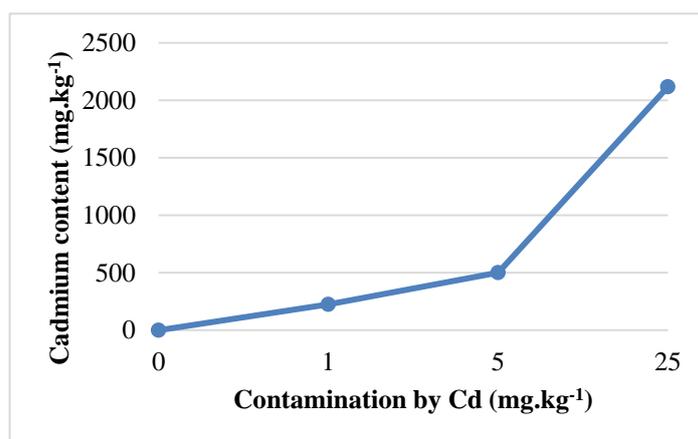
**Figure 1** Detail of the adaxial (upper) side of spring barley (*Hordeum vulgare* L.) leaf on an area of 1 mm<sup>2</sup>.



**Figure 2** Detail of the abaxial (lower) side of spring barley (*Hordeum vulgare* L.) leaf on an area of 1 mm<sup>2</sup>.



**Figure 3** The evaluation of cadmium content in the roots and leaves of barley.



**Figure 4** The evaluation of cadmium content in the barley.

The content of cadmium in leaves varied over a value in first variant  $5.57 \pm 0.29 \text{ mg.kg}^{-1}$ , in second variant  $11.68 \pm 2.14 \text{ mg.kg}^{-1}$  and in third variant  $52.93 \pm 6.73 \text{ mg.kg}^{-1}$  of dry mass.

The cadmium content in different parts of the plant increases proportionally with an increasing application of

heavy metal. The cadmium content in the root system was up to 40 times higher than the aboveground part of barley.

The bioaccumulation factor has been used as an effective way to show the potential of the plants for phytoremediation. It is the indicator of the ability of metal accumulation by plants. A good accumulator plant should

**Table 3** Cadmium effect on the number of leaf stomata in the central part of the barley leaf on adaxial (upper) and abaxial (lower) side.

Contamination by Cd (mg.kg <sup>-1</sup> )	Number of leaf stomata per 1mm <sup>2</sup> (adaxial side)	Number of leaf stomata per 1mm <sup>2</sup> (abaxial side)
0	59	51
1	53	58
5	59	63
25	60	67

**Table 4** Bioaccumulation factor and the translocation factor of barley according to contamination by cadmium.

Contamination by Cd (mg.kg <sup>-1</sup> )	BF of the roots (%)	BF of the leaves (%)	TF of the barley x 100
0	-	-	-
1	219.39	5.57	2.54
5	97.88	2.34	2.39
25	82.57	2.12	2.56

have a bioaccumulation factor lower than 100%. The translocation factor describes in which part of plant body is the highest accumulation of contaminant (Kherbani et al., 2015).

The bioaccumulation factor was calculated as:

$$BF = \text{Cd content in the plant} / \text{Cd content in the soil.}$$

The translocation factor was described as:

$$TF = \text{Cd content in the leaves} / \text{Cd content in the roots.}$$

In our case, the maximum bioaccumulation factor of roots was obtained with the value 97.88% to a 5 mg.kg<sup>-1</sup> for Cd.

The cadmium content in the leaves of barley was much lower than in the roots. Translocation factor is too small, so spring barley is very interesting plant for phytoextraction. The results are shown in Table 4.

In acid soils, cadmium is more mobile and less able to return the adsorption to sediments and minerals, rocks and sand. Adsorption of cadmium depends on its concentration, pH of the soil solution, soil type, duration of exposure and the concentration of complexing ligand. Cadmium is an element that is highly mobile in acidic soil. The mobility increases with decreasing pH, fertilizing by acid fertilizers and low content of organic matter in the soil (Trebichalský et al., 2010).

Phytoextraction of heavy metals is a technology that has been studied for several years. It is more ecological and cheaper way how to clean our environment.

Several plant species are known because they hyperaccumulate a high contents of metals from the soil, they are able to store particularly high amounts of heavy metals in aboveground organs. The accumulators are mainly herbaceous species, crops and nowadays angiosperm trees with a high growth such as poplars or willows. Woody species now represent attractive models since they have a higher biomass and a more important root system to decontaminate soils deeper than herbaceous plants (Saladin, 2015).

## CONCLUSION

Periodical monitoring of plants should be encouraged especially crops from areas that are grown and harvested next to the mining or industrial areas and the geochemical anomalies. The heavy metals may enter the leaves via the stomata.

The effect of contamination by cadmium to germination, length of leaves and number of stomata on abaxial side of leaf was confirmed. The contaminated soil by cadmium do not pose a risk of heavy metal entry into the feed and food chain by spring barley (*Hordeum vulgare* L.).

## REFERENCES

- Akpor, O. B., Ohiobor, G. O., Olaolu, T. D. 2014. Heavy metal pollutants in wastewater effluents: Sources, effects and remediation. *Advances in Bioscience and Bioengineering*. vol. 2, no. 4, p. 37-43. <http://dx.doi.org/10.11648/j.abb.20140204.11>
- Akher, M. F., Macfie S. M. 2012. Species-Specific relationship between Transpiration and Cadmium Translocation in Lettuce, Barley and Radish. *Journal of Plant Studies*. vol. 1, no. 1, p. 1-13. <http://dx.doi.org/10.5539/jps.v1n1p2>
- Akher, M. F., Omelon, Ch. R., Gordon, R. A., Moser, D., Macfie S. M. 2014. Localization and chemical speciation of cadmium in the roots of barley and lettuce. *Environmental and Experimental Botany*. vol. 100, no. 1, p. 10-19. <http://dx.doi.org/10.1016/j.envexpbot.2013.12.005>
- Wani, A. S., Ahmad, A., Hayat, S., Fariduddin, Q. 2013. Salt-induced modulation in growth, photosynthesis and antioxidant system in two varieties of Brassica juncea. *Saudi Journal of Biological Sciences*, vol. 20, no. 2, p. 183-193. <http://dx.doi.org/10.1016/j.sjbs.2013.01.006> PMID:23961235
- Cohen, C. K., Fox, T. C., Garvin, D. F., Kochian, L. V. 1998. The role of iron-deficiency stress responses in stimulating heavy-metal transport in plants. *Plant Physiology*. vol. 116, p. 1063-1072. <http://dx.doi.org/10.1104/pp.116.3.1063>
- Gardea-Torresdey, J. I., Peralta-Videa, J. R., Rosa, G. D., Parsons, J. G. 2005. Phytoremediation of heavy metals and

study of the metal coordination by X-ray absorption spectroscopy. *Coordination Chemistry Reviews*. vol. 249, no. 17-18, p. 1797-1810. <http://dx.doi.org/10.1016/j.ccr.2005.01.001>

Hoagland, D. R., Arnon, D. I. 1950. The water – culture method for growing plants without soil. *California Agricultural Experiment Station Circular*, vol. 347, p. 1-32.

Ivanišová, E., Ondrejovič, M., Dráb, Š., Tokár, M. 2011. The evaluation of antioxidant activity of milling fractions of selected cereals grown in the year 2010. *Potravinárstvo*, vol. 5, no. 4, p. 28-33. <http://dx.doi.org/10.5219/163>

Kabata-Pendias, A., Pendias, H. 2001. *Trace Metals in Soils and Plants*, 2<sup>nd</sup> ed., CRC Press, Boca Raton, Florida, USA.

Kherbani, N., Abdi, N., Lounici, H. 2015. Effect of cadmium and zinc on growing barley. *Journal of Environmental Protection*, vol. 2, no. 2, p. 160-172. <http://dx.doi.org/10.4236/jep.2015.62018>

Kocková, M., Valík, L. 2011. Potential of cereals and pseudocereals for lactic acid fermentations. *Potravinárstvo*, vol. 5, no. 2, s. 27-40. <http://dx.doi.org/10.5219/127>

Lachman, J., Dudjak, J., Miholová, D., Kolihová, D., Pivec, V. 2004. Effect of cadmium stress on the uptake and distribution of microelements copper and zinc in plant parts of barley (*Hordeum sativum* L.). *Scientia Agriculturae Bohemica*, vol. 35, no. 3, p. 81-86.

Lachman, J., Kotíková, Z., Zámečníková, B., Miholová, D., Száková, J., Vodičková, H. 2015. Effect of cadmium stress on barley tissue damage and essential metal transport into plant. *Open Life Sciences*, vol. 10, no. 1, p. 30-39. <http://dx.doi.org/10.1515/biol-2015-0004>

Lasat, M. M., Pence, N. S., Garvin, D. F., Ebbs, S. D., Kochian, L. V. 2000. Molecular physiology of zinc transport in the Zn hyperaccumulator *Thlaspi caerulescens*. *Journal of Experimental Botany*. vol. 51, no. 342, p. 71-79. <http://dx.doi.org/10.1093/jexbot/51.342.71>

Maslin, P., Maier, R. M. 2000. Rhamnolipid-enhanced mineralization of phenanthrene in organic-metal co-contaminated soils, *Bioremediation Journal*, vol. 4, no. 4, p. 295-308. <http://dx.doi.org/10.1080/10889860091114266>

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*. vol. 59, no. 1, p. 651-681. <http://dx.doi.org/10.1146/annurev.arplant.59.032607.092911>

Ochonogor, R. O., Atagana, H. I. 2014. Phytoremediation of Heavy Metal Contaminated Soil by *Psoralea Pinnata*. *International Journal of Environmental Science and Development*, vol. 5, no. 5, p. 440-443. <http://dx.doi.org/10.7763/ijesd.2014.v5.524>

Pence, N. S., Larsen, P. B., Ebbs, S. D., Letham, D. L., Lasat, M. M., Garvin, D. F., Eide, D., Kochian, L. V. 2000. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proceedings of the National Academy of Sciences*, vol. 97, no. 9, p. 4956-4960. <http://dx.doi.org/10.1073/pnas.97.9.4956>

Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A., Forestier, C. 2002. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *The Plant Journal*. vol. 32, no. 4, p. 539-548. <http://dx.doi.org/10.1046/j.1365-3113x.2002.01442.x>

Piršelová, B., Lukáč, P., Dobroviczka, T., Mészáros, P., Libantová, J., Moravčíková, J., Matušiková, I. 2010. Vplyv iónov kadmia na vybrané morfológicko-fyziologické charakteristiky bôbu obyčajného cv. Aštar. (Effects of cadmium ions on selected morphological and physiological characteristics of faba bean cv. Aštar). *Potravinárstvo*, vol. 4,

special issue, p. 337-341. Available at: [http://www.potravinarstvo.com/dokumenty/mc\\_februar\\_2010/pdf/3/Pirselova.pdf](http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/3/Pirselova.pdf)

Puertas-Mejía, M. A., Ruiz-Díez, B., Fernández-Pascual, M. 2010. Effect of cadmium ion excess over cell structure and functioning of *Zea mays* and *Hordeum vulgare*. *Biochemical Systematics and Ecology*, vol. 38, no. 3, p. 285-291. <http://dx.doi.org/10.1016/j.bse.2010.02.001>

Saladin, G. 2015. Phytoextraction of heavy metals: The potential efficiency of conifers. *Soil Biology*. vol. 44, p. 333-353. [http://dx.doi.org/10.1007/978-3-319-14526-6\\_18](http://dx.doi.org/10.1007/978-3-319-14526-6_18)

Sharma, S. S., Kaul, S., Metwally, A., Goyal, K. C., Finkemeier, I., Dietz, K. J. 2004. Cadmium toxicity to barley (*Hordeum vulgare*) as affected by varying Fe nutritional status. *Plant Science*, vol. 166, no. 5, p. 1287-1295. <http://dx.doi.org/10.1016/j.plantsci.2004.01.006>

Sharma, S. S., Dietz K. J. 2008. The relationship between metal toxicity and cellular redox imbalance. *Trends in Plant Science*, vol. 14, no. 1, p. 43-50. <http://dx.doi.org/10.1016/j.tplants.2008.10.007>

Stanbrough, R., Chuabonmee, S., Palombo E. A., Malherbe, F., Bhave, M. 2013. Heavy Metal Phytoremediation Potential of a Heavy Metal Resistant Soil Bacterial Isolate, *Achromobacter* sp. Strain AO22. *APCBEE Procedia*, vol. 5, p. 502-507. <http://dx.doi.org/10.1016/j.apcbee.2013.05.085>

Trebichalský, P., Molnárová, J., Musilová, J., Bystrická, J., Bajčan, D. 2010. Obsah kadmia a olova v zrne jačmeňa siateho s ohľadom na dodržanie ekologickej rovnováhy pestovateľského prostredia. (Cadmium and lead contents in barley grains from the view point of ecological balance cultivation environment). *Potravinárstvo*, vol. 4, special issue, p. 358-363. Available at: [http://www.potravinarstvo.com/dokumenty/mc\\_februar\\_2010/pdf/3/Trebichalsky.pdf](http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/3/Trebichalsky.pdf)

Tyokumbur, E. and Okorie, T. 2014. Toxic trace metal contamination (Arsenic, Cadmium and Lead) of *Sarotherodon melanothron* (Rupell, 1852) from Alaro stream in Ibadan. *Journal of Food and Nutrition Sciences*, vol. 2, no. 6, p. 258-261. <http://dx.doi.org/10.11648/j.jfns.20140206.13>

Wuana, R. A., Okieimen, F. E. 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *ISRN Ecology*. vol. 2011, p. 1-20. <http://dx.doi.org/10.5402/2011/402647>

Zhang, H. J., Dong, H. Z., Li, W. J., Zhang, D. M. 2011. Effects of soil salinity and plant density on yield and leaf senescence of field - grown cotton. *Journal of Agronomy and Crop Science*. vol. 198, no. 1, p. 27-37. <http://dx.doi.org/10.1111/j.1439-037x.2011.00481.x>

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