

## HYPERACCUMULATION OF CADMIUM IN MAIZE PLANT

*(Zea Mays)*

ALINA STINGU, IRINA STANESCU,\* IRINA VOLF and VALENTIN I. POPA

“Gheorghe Asachi” Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection, Department of Pulp and Paper, Iasi, Romania  
 \*“Anastasiu Fătu” Botanical Gardens, “Alexandru Ioan Cuza” University of Iasi, Romania

Received October 21, 2010

Maize plant responses, in terms of growth and metal uptake, to different concentrations of cadmium ions (4, 20  $\mu\text{M}$ ) were analyzed in a hydroponic culture, for 2 weeks. For a 4  $\mu\text{M}$  cadmium-contaminated environment, the maize plant presents the highest bioaccumulation level after 192 h, with a recovery degree of 52%, meanwhile, at a 20  $\mu\text{M}$  concentration, the highest bioaccumulation was registered after 366 h, with a corresponding recovery degree after 288 h (10.56%). The translocation factor presented higher values for 20  $\mu\text{M}$  induced contamination than for 4  $\mu\text{M}$ , which means that increasing metal concentration in the medium increased the concentration in the upper parts of the plant. Anatomical sections of a maize plant (in a 4 and 20  $\mu\text{M}$  cadmium-contaminated environment) were observed to evidence the changes in plant morphological structure. The efficiency of phytoextraction is related to the metal concentration in the environment and to the plant's ability to grow on polluted soil sites, concomitantly with a high biomass yield.

**Keywords:** heavy metal, phytoremediation, bioaccumulation, translocation factor

**INTRODUCTION**

Heavy metal contamination is a serious environmental problem that limits crop production and threatens human health through the food chain.

Cadmium, one of the most toxic environmental pollutants for plants, may interfere with numerous biochemical and physiological processes – including photosynthesis, respiration, nitrogen and protein metabolism, and nutrient uptake. Phytoremediation is an *in situ* nondestructive technique, characterized by the utilization of hyperaccumulator plant species to remove the heavy metals from soil.

The suitability of a certain plant for heavy metal remediation is determined by various plant properties, such as heavy metal tolerance, size, growth rate and rooting depth, heavy metal accumulation in above-ground plant parts and climatic adaptation and pest resistance.<sup>1,2</sup>

The aim of this research was to evaluate the maize plant responses to cadmium stress conditions, every 48 h, for 2 weeks, and the efficiency in phytoremediation processes.

**EXPERIMENTAL**

Maize seeds (*Zea mays*) were sterilized in the commercial bleaching agent HOCl (1%) for 30 min and rinsed with distilled water under stirring for 10 min, the process being repeated 3 times. The seeds were placed over moist filter paper disks in Petri dishes and stored in the dark at 25 °C, with a view to their germination.

The hydroponic units consisted of plastic pots containing a Hoagland nutrient solution (1 mM  $\text{KH}_2\text{PO}_4$ , 5 mM  $\text{KNO}_3$ , 5 mM  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 2 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 11.8  $\mu\text{M}$   $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.7  $\mu\text{M}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.32  $\mu\text{M}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.16  $\mu\text{M}$   $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , 46.3  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 5  $\mu\text{M}$  Fe) with the pH adjusted<sup>3</sup> to 6.8. The plastic pots were covered with an aluminum foil to prevent the development of photosynthetic algae. After 5 days of germination, seedlings of maize with the same size were assembled in each hydroponic unit. The volume of nutrient solution (150 mL) was not modified throughout the experiments, to avoid the variation of metal concentrations.

Every 48 h and at the end of the assay (2 weeks), the contents of cadmium in the roots, stem and leaves, as well as the growth parameters of maize plants, were determined. The plant roots were rinsed in abundant tap and distilled water

before mineralization. Maize plants separated into roots, stems and leaves were oven-dried at 60 °C, until constant mass was reached, and then the plant tissues were digested<sup>4</sup> using HNO<sub>3</sub> (65%) and H<sub>2</sub>O<sub>2</sub> (30%), on a hot plate at 120 °C, for at least 5 h. The measurement of the metal content in the solution was accomplished through AAS (using a GBC Avanta 2003 Atomic Absorption Spectrophotometer).

To evaluate the growth rate of the maize plant every 48 h, in a cadmium-contaminated environment, the following formula was used: growth rate, % = 100 x (growth parameters at the beginning of the experiment – growth parameters at a considered time)/growth parameters at a considered time.

Spectrophotometric quantification of heavy metal concentration in maize plant tissues permitted the evaluation of cadmium bioaccumulation, translocation factor and recovery:

Bioaccumulation coefficient = (cadmium concentration µg/g dry plant tissue)/(cadmium concentration µg/mL nutrient solution);<sup>5</sup>

Translocation factor (TF) = ratio of metal concentration in shoots/ratio of metal concentration in roots;<sup>6</sup>

Recovery, % = metal content in shoot or root/metal content in the medium.<sup>7</sup>

At the end of the experiment, histological cross-sections were obtained for maize roots. The sections were cut manually, using microtome and elder pith as a support. The histological sections were washed in sodium hypochlorite, then in acetic acid (to eliminate the cellular content) and distilled water. The sections were coloured with iodine green (1 min), washed in 90% ethylic alcohol and distilled water, then coloured with ruthenium red (1 min) and again washed in

distilled water.<sup>8</sup> In order to obtain the permanent slides, the histological sections were included in glycerol-gelatine drops, then analyzed on a KRÜSS light microscope. Light micrographs were performed with a Canon A540 camera. Bar = 100 µm.

## RESULTS AND DISCUSSION

In the first hours, under 4 µM cadmium stress conditions, an increasing trend in plant growth and development was observed, the maximum growth rate being registered at 144 h. 192 h after the beginning of the experiment, the growth process seemed to stop, being resumed after 48 h. For a 20 µM cadmium-contaminated environment, the maximum growth rate was registered in the first 48 h. After 192 h, a decreasing trend in plant growth was observed. Maize plant growth rate decreased with increasing cadmium concentration in the growth medium (Fig. 1).

Cadmium concentration (µg/g dry mass) and content (µg/plant) in maize plant presented different values, as depending on metal contamination level. The highest values for cadmium concentration and content (390.04 µg/plant), under 4 µM stress conditions (15430.99 µg/g), were registered at 192 h. Reported to the 20 µM cadmium-contaminated environment, the highest metal content (395.82 µg/plant) and concentration (22443.54 µg/g) were recorded at 228 and 336 h. These results could be correlated with the plant growth rate (Table 1).

Table 1  
Root, stem, leaf concentration (µg/g dry mass) and content (µg/plant) of cadmium in maize plant (*Zea mays*)

Cadmium concentrations	Time (h)	Concentration (µg/g dry weight)				Content (µg/plant)			
		Root	Stem	Leaf	Total/plant	Root	Stem	Leaf	Total/plant
4 µM CdCl <sub>2</sub>	48	4634.68	4448.87	1411.92	10495.48	114.94	80.08	46.17	241.19
	96	4515.53	4494.21	1994.41	11004.16	114.24	110.56	83.37	308.17
	144	4383.80	4496.90	1747.29	10628.00	131.08	43.62	91.73	266.43
	192	4265.07	10050.70	1115.21	15430.99	136.06	199	54.98	390.04
	240	3383.82	2657.97	126.53	6168.34	128.59	91.43	55.68	275.70
	288	4057.21	4660.84	1164.06	9882.12	143.63	109.09	56.57	309.26
	336	7675.96	3724.23	970.80	12370.99	115.14	94.22	53.69	263.05
20 µM CdCl <sub>2</sub>	48	2702.53	4264.63	2813.12	9780.29	123.51	90.84	78.49	292.83
	96	4721.85	6873.60	2600.82	1419.28	140.24	125.10	120.42	385.76
	144	4045.97	7501.24	3709.84	15257.06	149.70	120.02	117.23	389.95
	192	5091.14	6053.65	2215.58	13370.38	141.53	110.36	99.70	351.59
	240	3715.81	9138.62	2118.28	14972.72	149.00	125.00	90.24	364.44
	288	6221.26	11103.12	3763.57	21087.96	161.75	135.46	98.61	395.82
	336	10085.58	8867.51	3490.44	22443.54	136.16	118.82	47.47	302.45

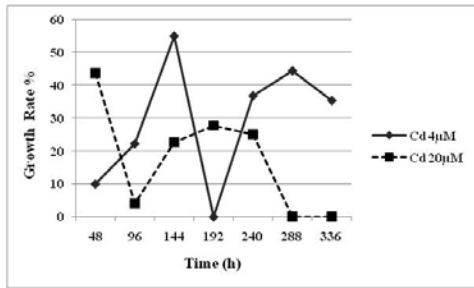


Figure 1: Growth rate of maize plant under cadmium stress conditions for two weeks

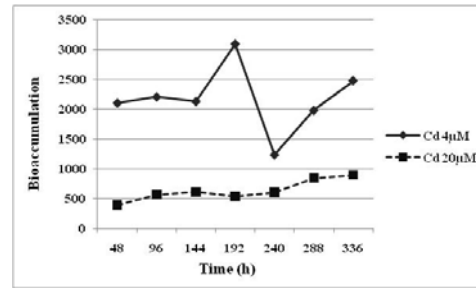


Figure 2: Cadmium bioaccumulation monitored in maize plant for two weeks

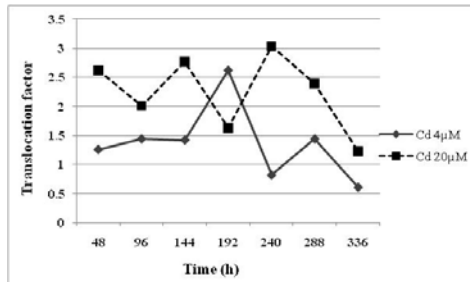


Figure 3: Translocation factor monitored in maize plant under cadmium stress for two weeks

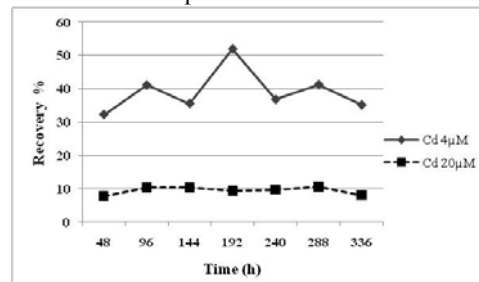


Figure 4: Recovery, %, monitored in maize plant under cadmium stress for two weeks

The bioaccumulation coefficient is characterized by lower values of 20  $\mu\text{M}$  cadmium concentration. For both cadmium contamination levels, in the first 144 h, an increasing trend in metal bioaccumulation was observed in the maize plant. The highest cadmium bioaccumulation level (4  $\mu\text{M}$   $\text{CdCl}_2$ ) was registered at 192 h (3086.20) and, for 20  $\mu\text{M}$  cadmium stress conditions, at 336 h (897.74) (Fig. 2).

The uptake of cadmium ions and translocation (2.62) to the aerial parts of maize plant, under 4  $\mu\text{M}$  metal treatments, were obvious at 192 h while, for the highest cadmium level, the translocation factor showed the maximum values (3.03) at 240 h (Fig. 3).

Maize plant recovery (%) was more efficient in a 4  $\mu\text{M}$  cadmium-contaminated environment, the maximum recovery being observed at 192 h (52%). If increasing cadmium concentration in the growth medium, a relatively constant recovery capacity (10%) of the maize plant was recorded along the two weeks (Fig. 4). Anyway, obtaining a recovery percentage of 10% under 20  $\mu\text{M}$  cadmium stress conditions allows the assumption that the maize plant is suitable for bioremediation, and could be properly used as an efficient hyperaccumulator plant, as long as the literature considers that the hyperaccu-

mulator plants contain more than or up to 0.01% cadmium by dry mass.<sup>9,10</sup>

The cross-sections of the root, carried out at the end of the experiment for all three tested solutions, provided no visible difference between the control sample and the samples contaminated with cadmium (4  $\mu\text{M}$  and 20  $\mu\text{M}$  contamination). The maize root cross-sections (40x and 400x) are presented in Figure 5. Histological analysis permitted to observe the following anatomic regions: rhizodermis, cortex and central cylinder. Rhizodermis is single-layered, formed by small cells with thin walls; the cortex presents a thick cortical parenchyma (8-10 layers) with thin cellulosic walls disorganized in a few parts, aeriferous channels thus resulting. The central cylinder is well-structured. Xylemic and phloemic vascular bundles can be observed. The xylemic bundles present 1-3 vessels of protoxylem, with cellulose walls and 1 metaxylem vessel with cellulose walls too, towards the center of the cylinder. The center of the root is represented by pith, which contains cells with thin-cellulosed walls. As long as approximately the same structures were observed for the two contamination levels, comparatively with the control, it may be ascertained that maize plant tolerated higher amounts of cadmium, being therefore suitable for phytoremediation processes.

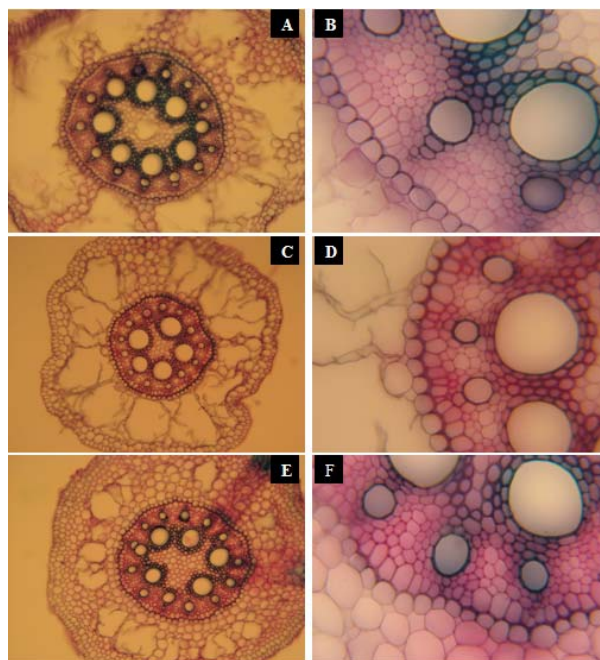


Figure 5: Cross-sections of *Zea mays* root: A – control sample (distilled water), 40x, B – control sample (distilled water), 400x, C – 4  $\mu\text{M}$  Cd contamination level in the growth medium, 40x, D – 4  $\mu\text{M}$  Cd contamination level in the growth medium, 400x, E – 20  $\mu\text{M}$  contamination level in the growth medium, 40x, F – 20  $\mu\text{M}$  contamination level in the growth medium, 400x

## CONCLUSIONS

Maize plant responses to cadmium stress depended on cadmium concentration in the growth medium.

For 4  $\mu\text{M}$  cadmium concentrations, the best recovery (%), translocation factor and metal bioaccumulation by the maize plant were obtained after 192 h from the beginning of the experiment. At the same time, no growth was registered.

For a 20  $\mu\text{M}$  metal contamination level, the efficiency of cadmium concentration and bioaccumulation was registered at the end of the experiment, after 336 h, when the growth process slowed down. Recovery (%) presented a linear trend of 10% at every 48 h.

The maize plant could be properly used in heavy metal bioremediation processes, due to the considerable biomass production, metal bioaccumulation and translocation to the aerial part and recovery (%), which presented significant values.

**ACKNOWLEDGEMENTS:** The study was supported by the “BRAIN Doctoral Scholarships as an investment in intelligence” projects, financed by the European Social Found and by the Romanian Government.

## REFERENCES

- <sup>1</sup> M. B. Kirkham, *Geoderma*, **137**, 19 (2006).
- <sup>2</sup> K. Jensen, P. E. Holm, J. Nejrup, B. L. Morten, K. Ole and J. Borggaard, *Environ. Pollut.*, **157**, 931 (2009).
- <sup>3</sup> D. R. Hoagland and D. I. Aron, California Agricultural Experiment Station, Circular 347, College of Agriculture, University of California, Berkeley, 1950.
- <sup>4</sup> P. G. Smith, I. Koch and K. J. Reimer, *Sci. Total Environ.*, **390**, 188 (2008).
- <sup>5</sup> A. Singh, S. Eapen and M. H. Fulekar, *Rom. Biotech. Lett.*, **14**, 4164 (2009).
- <sup>6</sup> Y. Sun, Q. Zhou, W. Tao, A. Liu Jing, Z. Xu and W. Lin, *J. Hazard. Mater.*, **165**, 1023 (2008).
- <sup>7</sup> R. Hajiboland, *Plant Soil Environ.*, **51**, 156 (2005).
- <sup>8</sup> M. Andrei and R. M. Paraschivoiu, in “Microtehnica botanică” (in Romanian), Niculescu Publishing House, București, 2003, pp. 44-54.
- <sup>9</sup> R. D. Reeves and A. J. M. Baker, in “Phytoremediation of toxic metals: Using plant to clean up the environment”, edited by I. Raskin, B. D. Ensley, Wiley, New York, 2000, pp. 193-230.
- <sup>10</sup> A. Stingu, I. Volf and V. I. Popa, *Environ. Eng. Manag. J.*, **8**, 1247 (2009).