JASEM ISSN 1119-8362

Full-text Available Online at www.bioline.org.br/ja

J. Appl. Sci. Environ. Mgt. *March*, 2006
Vol. 10 (1) 83 - 87

Effects of heavy metals on some proximate composition of *Eichhornia crassipes*

[1]* ODJEGBA, V J; [2]FASIDI, I O

[1]Department of Botany and Microbiology, University of Lagos, Akoka, Yaba Lagos, Nigeria. odjesvic@yahoo.com
Tel: 234- 8023323406

[2]Department of Botany and Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria.

ABSTRACT: Effects of 8 heavy metals (Ag, Cd, Cr, Cu, Hg, Ni, Pb and Zn) on the total chlorophyll, foliar proline, and protein and starch contents of *Eichhornia crassipes* was investigated. Plantlets were grown in quarter-strength Hoagland's solution and supplemented with 0, 0.1, 0.3, 0.5, 1.0, 3.0 and 5.0 *mM* of each of the metals for 3 weeks. There was a significant reduction ($p \leq 0.05$) in total chlorophyll, protein and starch contents, while foliar proline increased significantly. These effects were however dependent on the nature of the metal, its concentration and duration of exposure. @JASEM.

Among the variety of substances entering the soil, inland waters and the ocean as waste products, heavy metals especially create long term problems. Not only do they accumulate in organisms, and thus circulate in food chain, they also remain in the ecosystem in dangerous concentration for longer period in sediments (Ma *et al*., 1997). Toxic levels of some heavy metals appear as a result of environmental pollution due to the removal technology of mining, heavy vehicular traffic, smelting, manufacturing, and agricultural wastes in natural and agricultural areas (Oncel *et al*., 2000). The toxicity of these heavy metals to plants varies with individual metal and concentrations. Induction of leaf chlorosis and reduction of biomass production have been observed on crops grown in soils contaminated with moderate levels of heavy metals (Clijsters *et al*., 1999). The increasing levels of heavy metals in the environment, their entry into the food chain, and the overall health effects are of major concern to researchers in the field of environmental biology. To this end, recent years have witnessed a flurry of research activities (in advanced nations) concerning pollution caused by trace elements. Despite this positive development, the effects of trace elements on plants are poorly understood in Nigeria as only limited data exist and they are not readily available. In view of the scanty reports regarding the effects of trace elements on aquatic flora found in Nigerian waters, this work reports the effects of 8 heavy metals on the proximate composition of *Eichhornia crassipes*, in other to contribute to the existing knowledge regarding this subject in Nigeria.

MATERIALS AND METHODS

Eichhornia crassipes were collected from Oba dam, University of Ibadan, Ibadan, Oyo State. Plants were washed thoroughly under a running tap water and were grown and propagated for 4 weeks in quarter-strength Hoagland's solution (Hoagland and Arnon, 1938), containing (*mM*): 1.25 $Ca(NO_3)_2$, 1.5 KNO_3, 0.5 KH_2PO_4, 0.5 $MgSO_4$ and 0.25 NaCl, and (μM): 11.5 H_3BO_3, 2.3 $MnCl_2$, 0.026 H_2MoO_4 and 11.2 FeEDTA. Plants of similar size were selected for the experiment. The trace elements under study were supplied at 0.1, 0.3, 0.5, 1.0, 3.0 and 5.0*mM* as $AgNO_3$ (Ag), $Cd(NO_3)_2$ (Cd), K_2CrO_4 (Cr), $CuSO_4$ (Cu), $HgCl_2$ (Hg), $NiSO_4$ (Ni), $Pb(NO_3)_2$ (Pb) and $ZnSO_4$ (Zn). Nutrient solution devoid of any of these trace elements served as control. Both the control and the treated solutions were maintained at pH 5.5 using dilute HCl or NaOH. Experimental plants (in triplicates) were placed in nutrient solution (1 litre) supplemented with one of the trace elements under investigation and was replicated three times. Solutions were replenished every 5 days to prevent depletion of metals and nutrients. The experimental set up was maintained for 21 days in a screenhouse, nursery section of the Department of Botany and Microbiology, University of Ibadan. Harvested plants were washed in running tap water and rinsed with deionized water.

Extraction and estimation of chlorophyll was done using the method of Maclachlam and Zalik (1963) as described by Singh and Rao (1981). Fresh leaves (3.0g) from each of the replicates representing each treatment were separately ground in mortar containing small amount of sodium trioxocarbonate iv (Na_2CO_3) in order to keep the chlorophyll structure intact. Extracts were made with 25ml 80% acetone and filtered through labsman no.1 filter paper. Extracts were centrifuged at 15000g for 20 minutes using IEC model k-centrifuge. Extracts devoid of residue were used for spectrometer readings. Carbohydrate was determined according to method by Hansen and Moller (1975) with slight modifications. Ground dry leaves (2g) were

E-mail: odjesvic@yahoo.com
Tel: 234- 8023323406

separately dissolved in 30ml 80% ethanol at $85^0$for 5 minutes to extract free carbohydrate. Filtration was done using labsman no.1 filter paper. The filtrate was kept in a refrigerator, while the residue was refluxed for 5 minutes with 30ml 0.1*M* HCl. After cooling, it was neutralized with NaOH. The solution was mixed with the stored filtrate. A volume of 15ml 0.3*M*. $Ba(OH)_2$ and 15ml $ZnSO_4.7H_2O$ were added to the solution to precipitate protein contained in the sample. It was centrifuged at 10000g for 10minutes. The supernatant was transferred into 100ml test tube using Pasteur pipette, and was made up to mark with distilled water. The solution (2ml) was transferred into a test tube and 4ml anthrone-H_2SO_4 reagent (0.2g anthrone + 100ml conc. H_2SO_4) was added. The mixture was allowed to boil in a water bath for 10 minutes. The mixture was cooled to room temperature, and absorbance read at 620nm using corning 258 spectrophotometer. A mixture of distilled water and anthrone reagent served as blank. The glucose content was determined from a standard curve, and the amount of starch was calculated by multiplying its glucose content by 0.9 (Nakano *et al*., 2000). Dried ground leaves (0.5g) were used for the proline extraction and its determination. This was carried out according to the method described by Bates *et al.,* (1973). The amount of protein was determined using the modified Kjedahl method for the estimation of total organic nitrogen in the dried plant samples as described by Eastin (1978). The nitrogen value in the sample was multiplied by 6.25, to obtain the amount of protein (Ramalho *et al*., 2000).

Statistical analyses: Data analyses were performed using SAS version 6.0 for personal computers (SAS Institute, 1989). For mean separations, Duncan's multiple range test (DMRT) values were used at $P \leq 0.05$.

RESULTS AND DISCUSSION

The mean chlorophyll content in leaves of control *E. crassipes* was 25.73±0.02 mg g^{-1} fresh weight. A slight increase in chlorophyll level was observed when *E. crassipes* was exposed to 0.1*mM* of these metals. With the exception of Zn, 0.3*mM* of the metals investigated induced a gradual decrease in the chlorophyll content (Fig 1).

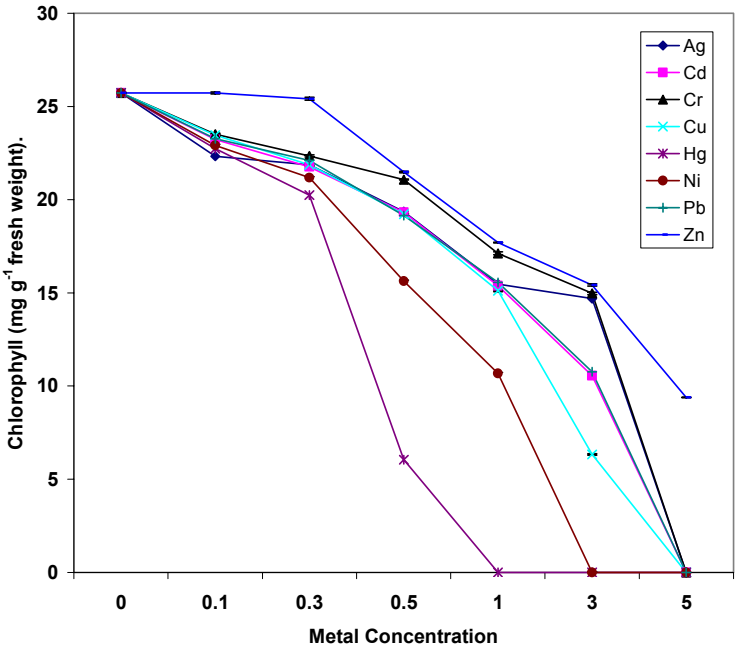

Figure 1: Chlorophyll content (mg g^{-1} fresh weight) of *E. crassipes* grown in nutrient medium supplemented with different concentrations (*mM*) of heavy metals.

The result agreed with the findings reported by Monni *et al*., (2001) that the total chlorophyll in the leaves of *Empetrum nigrum* (crowberry) growing close to Cu and Ni smelter decreased significantly. Many deleterious environmental influences that inhibit plant growth, ranging from nutrient deficiencies to anthropogenic pollution, can result in decreased leaf chlorophyll contents (Hendry *et al*., 1987, Carter and Spiering, 2002). In turn, this could

have a direct consequence on starch and protein contents of the plant.

Data regarding the starch contents show significant changes resulting from treatments of heavy metals. The starch contents of the control plants were significantly ($p \leq 0.05$) more than that recorded for metal treated plants, especially at prolonged exposure period. The starch content of the control *E. crassipes* at the end of the experiment was 77.20±0.02 mg g^{-1} dry weight. A value that is significantly ($p \leq 0.05$) greater than the respective values recorded for 0.3*mM* Ag, Cd, Cr, Cu, Ni, Hg, Pb and Zn which were 73.71±0.04, 73.69±0.06, 74.01±0.02, 73.67±0.03, 70.09±0.01, 72.16±0.02, 73.68±0.02 and 74.62±0.00 mg g^{-1} dry weight (Figure 2).

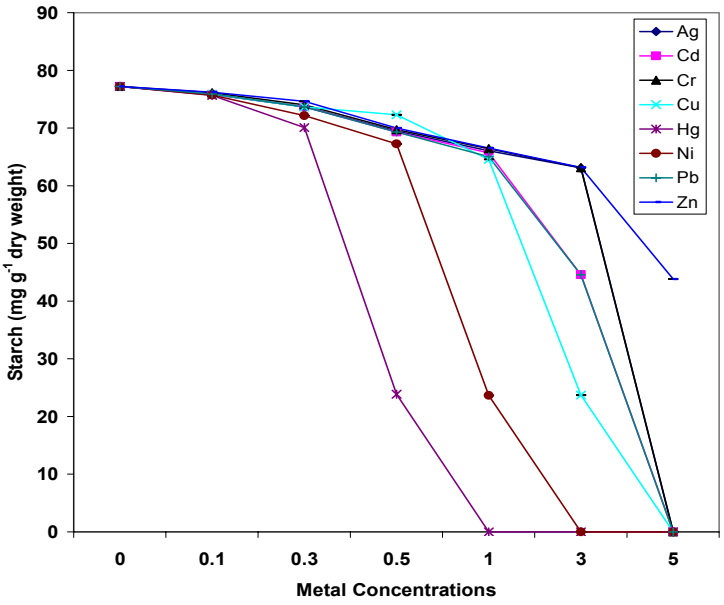

Figure 2: Starch content (mg g^{-1} dry weight) of *E. crassipes* grown in nutrient medium supplemented with different concentrations (*mM*) of heavy metals.

The decrease in the total starch contents could be as result of the direct consequence of chlorotic leaves, interference with the photosystems and inhibition of the Calvin cycle enzymes (Clijsters *et. al.,* 1999).Results regarding the foliar proline show significant changes resulting from treatment of heavy metals. The changes depending on the metal, their various concentrations and the duration of treatments are shown as a percentage of the control (Table 1).

Table 1: Percentage increase in foliar proline in *E. crassipes* exposed to different concentrations (*mM*) of heavy metals. Values with different letters in the same column indicate significant difference at $p \leq 0.05$, according to Duncan's multiple range test (DMRT).

Metals / Conc.	Ag	Cd	Cr	Cu	Hg	Ni	Pb	Zn
0.1	43.65e	58.58e	33.34f	46.87e	123.05c	104.62d	43.94f	17.99f
0.3	100.75d	120.37d	61.13e	104.69d	262.68b	222.93c	83.26e	39.28e
0.5	154.13c	161.29	84.73d	138.10c	289.45a	351.35b	134.38d	67.39d
1.0	233.65b	234.96	218.57c	244.94b	*	404.60a	188.94c	196.32c
3.0	330.71a	269.94	325.65b	274.71a	*	*	270.25b	307.97b
5.0	*	*	347.83a	*	*	*	314.09a	355.46a

Eichhornia crassipes exposed to low concentrations of metals for a short period had lower proline contents in their leaves compared to those treated with higher concentrations and a longer period. The differences between the means of the foliar proline contents were statistically significant ($p \leq 0.05$). For each metal, the proline content increased rapidly with increase in concentration and treatment duration. There was also a significant difference between means of foliar proline when different metals were

compared at the same concentration. While 0.1*mM* Zn induced accumulation of foliar proline 17.99 %, 0.1 *mM* Hg induced foliar proline accumulation by 123.05 % (Table 1). Increased foliar proline levels likely acts as an antioxidant in metal-stressed cells. Proline reduces metal-induced free radical damage and maintains a more reducing environment (higher gluthione levels) in the cell (Siripornadulsil, 2002).

The protein levels of *E. crassipes* treated with different concentrations of heavy metals decreased gradually as the metal concentrations increased. The mean values of protein at a particular concentration varied between metals. After 3 weeks of exposure to 0.3*mM* of Ag, Cd, Cr, Cu, Hg, Ni, Pb, and Zn, 59.86±0.01, 59.84±0.02, 60.54±0.03, 57.83±0.00, 56.28±0.00, 57.09±0.02, 59.84±0.01 and 61.10±0.02 mg g^{-1} dry weight protein was observed respectively. While the protein contents of the control plants and those exposed to 0.1*mM* increased gradually with time, those of plants grown in nutrient medium containing ≥0.3*mM* of the metals (except 0.3*mM* Zn) gradually decreased as the exposure period progressed (Figure 3).

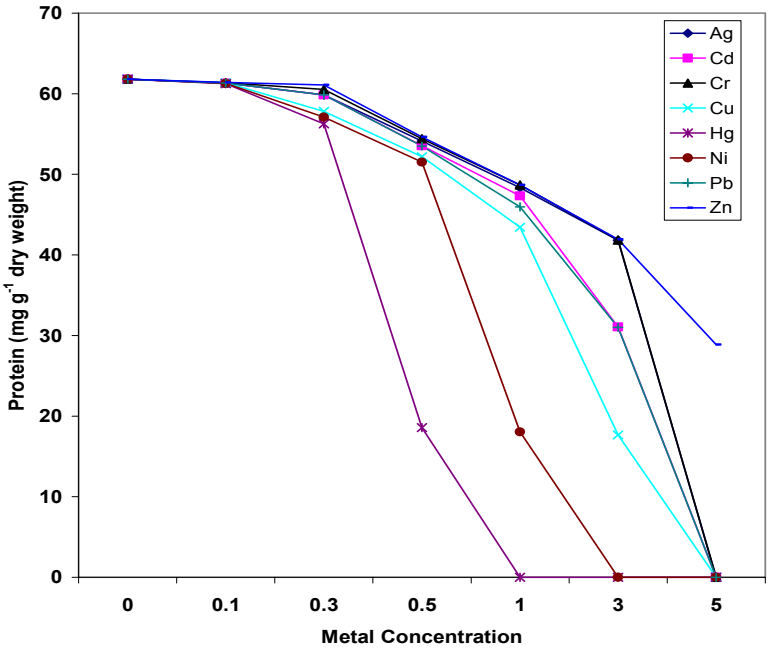

Figure 3: Protein content (mg g^{-1} dry weight) of *E. crassipes* grown in nutrient medium supplemented with different concentrations (*mM*) of heavy metals.

The poor protein formation could be related to disruption of nitrogen metabolism in this plant by the high doses of these metals. Since nitrogen is one of the primary essential nutrients involved as a constituent of biomolecules such as nucleic acids, nitrogen bases, coenzymes and proteins, any deviation in these constituents would inhibit the growth and yield of plants (Sharma *et al*., 1995). Overall, results from this study indicate the negative effects of trace elements on *Eichhornia crassipes*. Therefore, the increasing level of trace elements in our environment should be of serious concern in this part of the world both to the government and the general public. Also, the use of *Eichhornia crassipes* as a potential bioindicator of environmental quality especially when the leaves optical properties in the visible spectrum are considered is promising.

REFERENCES

Bates, LS; Waldren, RP; Teare, LD (1973) Rapid determination of free proline for water-stress studies. Plant and Soil, 39:205-207.

Carter, GA; Spiering, BA (2002) Optical properties of intact leaves for estimating chlorophyll concentration. Journal of Environmental Quality, 31:1424-1432.

Clijsters, H; Cuypers, A; Vangronsveld, J (1999) Physiological response to heavy metals in higher plants; Defence against oxidative stress. Zeitschrift fur Naturforsch, 54c:730-734.

Eastin, EF (1978) Total nitrogen determination for plant material containing nitrate. Analytical Biochemistry, *85:591*-594.

Hansen, J; Moller, I (1975) Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Analytical Biochemistry, 68:87-94.

Hendry, GAF; Houghton, JD; Brown, SB (1987) Tansley review no. 11: The degradation of chlorophyll – A biological enigma. New Phytologist, 107: 255-302.

Hoagland, DR; Arnon, DI (1938) The water culture method for growing plants without soil. *California Agriculture Experimental Station Bulletin,* 347, Berkeley, California, USA.

Ma, LQ; Tan, F; Harris, WG (1997) Concentrations and distributions of eleven metals in Florida soils. Journal of Environmental Quality*,* 26:769-775.

Maclachlam, S; Zalik, S (1963) Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. Canadian Journal of Botany, 41:1053-1062.

Monni, S; Uhlig, C; Hansen, E; Magel, E (2001) Ecophysiological responses of *Empetrum nigrum* to heavy metals pollution. *Environmental Pollution,* 112(2):121-129.

Nakano, H; Muramatsu, S; Makino, A; Mae, T (2000) Relationship between the suppression of photosynthesis and starch accumulation in the pod-removed bean. Australian Journal of Plant Physiology, 27:167-173.

Oncel, I; Kele, Y; Ustun, AS (2000) Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environmental Pollution, 107(3):315-320.

Ramalho, JC; Pons, TL; Groeneveld, HW; Azinheira, HG; Nunes, MA (2000) Photosynthetic acclimation to high light conditions in mature leaves of *Coffea arabica* L.: role of xanthophyll quenching mechanisms and nitrogen nutrition. Australian Journal of Plant Physiology*,* 27:43-51.

SAS Institute (1989) SAS procedures guide. Version 6. 3rd edition SAS Institute, Cary, NC. USA.

Sharma, DC; Chatterjee, C; Sharma, CP (1995) Chromium accumulation and its effects on wheat (*Triticum aestivum* L CV. HD 2204) metabolism. Plant Science, 111: 145-151.

Singh, SN; Rao, DN (1981) Certain responses of wheat to cement dust pollution. Environmental Pollution (series A) 24:75-81.

Siripornadulsil, S; Traina, S; Verma, DPS; Sayre, RT (2002) Molecular mechanism of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. Plant Cell, 14: 2837-2847

.