

## TREATMENT OF CADMIUM, COPPER, ZINC AND IRON IN WASTEWATER BY THE HORNWORT *CERATOPHYLLUM DEMERSUM*

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*( Received : Agust 14 ,1999 )*

**Key words:** *Ceratophyllum*, hornwort, treatment, wastewater.

### ABSTRACT

The hornwort, *Ceratophyllum demersum* L., was investigated as a potential biological filter for metal removal from domestic effluent. The plants were grown on media with a series of sewage dilutions. The waste water concentration of 2:5 was optimum for plant harvest realizing maximum uptake and accumulation of Cd and Cu within 9 days treatment. Their contents were, respectively, 2.9 and 3.9 folds higher than that of the control plants. Zinc was more readily accumulated with an increase of 2.2-75.5 times the initial content in the plant. Iron was greatest in plants grown on 4:5 waste dilution in 7 days treatment. Manganese ion increased slightly by the first waste treatment but was suppressed leakage by the successive runs, while  $K^+$  and  $Na^+$  ions loss was less pronounced at the beginning of treatment followed by considerable increase in  $K^+$  content. Growth rates did not exceed  $4.0g\ g^{-1}$  fresh weight  $d^{-1}$  at low waste dilutions. *Ceratophyllum*, may prove useful in the detoxification of domestic effluents, it has a capacity to retain heavy metals within its tissues

## INTRODUCTION

During the last two decades, heavy metals have become common contaminants of aquatic and wetland environments. Heavy metals can be found in domestic and industrial wastewaters. A significant input from domestic sources including materials used in distribution and plumbing networks, body care preparations, tap water and detergents. The secondary producers at the base of many aquatic food chains are vascular plants. A characteristic of the vascular plants found in aquatic and wetland environments is that they accumulate metals and other elements in excess of their physiological need. Several aquatic plants have been suggested for use in wastewater renovation systems. The uptake of heavy metals by *Eichhornia crassipes* Mart., *Salvinia molestra* Mitchell, *Spirodela polyrrhiza* Schleid, *Lemna minor* L. and *Azolla pinnata*. R.Br. has been reported by Chigbo *et al.* (1982), Jain *et al.* (1990) and Strivastav *et al.* (1994). The hornwort, *Ceratophyllum demersum* is among the submerged plants which best tolerates the progressing excessive eutrophication in water bodies, and it may even increase its area of occurrence at the expense of other communities of aquatic vegetation Voge (1986).

In the present study, experiments were conducted using *Ceratophyllum demersum* L. to assess its potential for Cu, Zn, Cd and Fe accumulation from domestic wastewater. The effect of contamination on growth rates and Mn, K and Na ions in the plant tissues were also investigated.

## MATERIALS AND METHODS

### Plant sample

Test plant, *Ceratophyllum demersum* L., was collected from a natural pond in the botanical garden in the Faculty of Science of

Alexandria. Selected fresh plants were transferred and maintained for a week in modified 0.10 M Hoagland solution as a pretreatment (Hoagland and Arnon, 1950)

### **Water samples**

Crude domestic sewage was sampled from the main sewer which collects wastewater from the western district of Alexandria. The samples were left to settle for 12 hours in big jars and the supernatant fluid was used for the culture experiments. Tap water was left in big containers for 3 days to get dechlorinated water.

### **Metal uptake experiment**

A series of dilutions of wastewater and chlorine free tap water to obtain concentrations 1:5, 2:5, 3:5 and 4:5 sewage were prepared. Fresh plants of about 2.5 g/L. medium were cultured in glass jars of 20 L. capacity. Three replicates were used for each dilution. All jars were exposed to air temperature and natural sunlight. The media were replenished at 7-10 days intervals, based on preliminary studies monitoring solution concentrations, maintaining the same dilutions. The plant yield was harvested, but keeping the same initial weight inoculum. The experiment lasted 28 days (with three incubation periods 9, 9 and 10 days) for media with 1:5 and 2:5 wastewater, but the media with higher dilutions lasted only 7 days and received only one doze of wastewater.

### **Metal analysis**

Harvested plant material was wrapped in paper towels and dried in an oven at  $70 \pm 2^\circ\text{C}$  to a constant weight. Dried plant was digested with concentrated nitric acid followed by perchloric acid. Trace metal levels and Mn, K and Na concentrations were carried out with Perkin-Elmer model 2380 atomic absorption spectrophotometer, using flame emission, against standard spectrosol metal solutions for atomic absorption analysis. The concentration of metal in *Ceratophyllum* (d) was obtained by the

equation:  $d=a \times b/c$  where a is the concentration of the metal in the diluted sample; b is the dilution factor and c is the dry weight of the sample (APHA, 1985).

## RESULTS AND DISCUSSIONS

Table (1) shows the concentrations of Cd, Cu, Zn and Fe in *Ceratophyllum* dry matter ( $\mu\text{g g}^{-1}$ ) after exposure to different wastewater dilutions.

**Cadmium.** The mean Cd concentration inoculated to the cultures of *Ceratophyllum* was  $0.3 \mu\text{g l}^{-1}$ . Cadmium accumulated in the plant tissues reached maximum after the second wastewater enrichment (18 days) in the lowest dilution (1:5) (Table 1). The highest Cd content was sustained after the first waste inoculum (9 days) in the other dilutions. However, Cd in the plants decreased thereafter in 1:5 and 2:5 concentrations by about 58.8 and 82% respectively. These results were almost approached by Sajwan and Harold (1994) for *Spirodela polyrrhiza* exposed to  $0.04\text{--}7.63 \mu\text{g Cd l}^{-1}$  solution, since it accumulated greatest Cd concentrations at one week followed by decreases through third and fourth weeks by 60-90%. This indicates a plant harvest regime should be a 9 days interval for maximum uptake of Cd from wastewater at 2:5 media dilution, then the replacement by new uncontaminated *Ceratophyllum* to carry out metal removal from waste effluent.

**Copper.** Copper concentration in sewage effluent had a mean value of  $26.6 \mu\text{g l}^{-1}$ . From initial Cu content in the plant dry weight of  $12.9 \mu\text{g g}^{-1}$ , it increased by 1.05 – 3.6 folds that in the control plants. The greatest Cu content was reached after 9 days incubation (first treatment) at 2:5 waste dilution (Table 1). Copper accumulated by *Ceratophyllum* was slightly lower than the world – wide mean value ( $48 \mu\text{g g}^{-1}$ ) reported by Hutchinson (1975), who suggested that this value in the leaves of aquatic plants does not cause harm.

Cadmium and copper levels accumulated by *Ceratophyllum* were still higher than estimated by Pip and Stepaniuk (1992) in above – and below ground portions of several macrophytes. These were; *Potamogeton* spp., *Glyceria* sp., *Nuphar* sp. and *Myriophyllum* sp., growing in 29 stream habitats which received run off from agricultural and industrial sewage, They accumulated between 0.01 and 4.94  $\mu\text{g g}^{-1}$  Cd and a maximum of 19.2  $\mu\text{g g}^{-1}$  Cu.

Zinc. Wastewater contained a mean Zn concentration of 160  $\mu\text{g l}^{-1}$ . From initial content of 12.9  $\mu\text{g g}^{-1}$  plant dry weight, increases fluctuating between 2.2 and 75.5 folds that in the control plant were reached . The greatest Zn accumulation ( 906  $\mu\text{g g}^{-1}$  ) was obtained by the end of the second treatment (18 days) at 2:5 media . Zinc content in *Ceratophyllum* decreased by additional as well as higher waste effluent concentrations. Its uptake might be inhibited by high concentrations of Cu and Cd, probably by inhibition of growth or by competition among the elements at uptake (Bryan, 1969).

Iron . *Ceratophyllum* was subjected to wastewater enriched with a mean Fe concentration of 32  $\mu\text{g l}^{-1}$  . Maximum Fe accumulated by the plant was sustained after 9 days incubation period ( first run ) at all dilution levels, with a highest value of 4291  $\mu\text{g g}^{-1}$  in plants grown on 4 : 1 dilution.

The results obtained in the present study indicates that *Ceratophyllum* efficiency for Zn and Fe uptake and accumulation from metal contaminated water exceeded that of *Azolla pinnata* , investigated by Rother and Whitton (1988). These authors reported from 48.0 to 101.9  $\mu\text{g g}^{-1}$  Zn and a maximum of 2710  $\mu\text{g g}^{-1}$  Fe in *Azolla* dry tissue after exposure to 0.3 – 0.055  $\text{mg l}^{-1}$  Zn and 0.410  $\text{mg l}^{-1}$  Fe during the time of peak flood season .

Generally, decreasing trends of Cd, Cu, Zn and Fe observed by the second and / or third treatments could be due to mobilization of metals particularly Cd and Zn (Sela *et al.*, 1988). However, concentrations are lowest in the younger parts of the plant as a result of continuing growth, therefore, their metal content in the whole plant will gradually fall. On the other hand, the variations in the metals uptake and accumulation by plants probably due to selectivity and metal concentration in the water as well as the metabolic activities of the test plant.

### **Toxic effect of heavy metals on the growth and essential cations in *Ceratophyllum***

*Ceratophyllum* could withstand the low sewage dilutions with yield rates between 3.5 and 4.0, 2.18 and 3.0 g g<sup>-1</sup> freshweight d<sup>-1</sup> obtained from media with 1:5 and 2:5 sewage respectively. The media contaminated with higher sewage dilutions survived for only a week with a yield of 2.75 g g<sup>-1</sup> d<sup>-1</sup> obtained from media with 3:5 dilution.

Table 2 shows the essential cations content in *Ceratophyllum*. High levels of copper, cadmium and zinc can be toxic to plants. Manganese is an essential micronutrient required for oxidation of water in photosynthesis and for detoxification of superoxide radicals (Burnell, 1988). Its content increased by 1.9 times in *Ceratophyllum* grown on low waste dilutions, but it dropped to 90 – 96% by additional waste effluent and at higher dilutions also. This could be due to that accumulated Cd and Zn antagonize Mn nutrition as Sunda and Huntsman (1996) indicated. Hart *et al* (1979) showed also that Cd competitively inhibited Mn uptake by *Chlorella pyrenoidosa* and was transported into the cell by Mn uptake system.

The amount of the alkali metals K<sup>+</sup> and Na<sup>+</sup> increased by 8.0 and 3.5 times, respectively, their initial in the control plant at the end of the third treatment at media with 2:5 waste effluent. Sodium ion generally

increased during the experiment at all media levels, while  $K^+$  fluctuated between a slight declination and a loss by about 48.5 at media with 3:5 waste water . Loss of  $K^+$  by 10-30% has been reported in *Azolla filiculoides* fronds grown on media containing 8-15 ppm of Cd , Cr , Cu and Zn according to Sela *et al* (1988).

### CONCLUSIONS

- 1- This study suggested that the aquatic vascular plant hornwort *Ceratophyllum demersum* L. could be a potential for cycling Cd, Cu , Zn and Fe in domestic wastewater.
- 2- The test plant indicated also tolerance to high load of sewage effluent, but with reduced biomass along the period of treatment and at higher sewage concentrations.

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Table 1. Concentrations of Cd, Cu, Zn and Fe in *Ceratophyllum demersum* L. ( $\mu\text{g g}^{-1}\text{d.w.}$ ) at the end of each incubation period. The control plant contained 2.09  $\mu\text{g Cd}$ , 12.9  $\mu\text{g Cu}$ , 12  $\mu\text{g Zn}$  and 1506  $\mu\text{g Fe}$ .

Wastewater Dilutions	Growth Periods (days)	Concentrations of metals ( $\mu\text{g g}^{-1}\text{d.w.}$ ) in <i>Ceratophyllum</i>			
		Cd	Cu	Zn	Fe
1:5	9	2.86	21.95	220	1645
	9	4.81	24.80	906	938
	10	1.98	13.59	398	626
2:5	9	6.02	46.60	434	2989
	9	1.08	22.40	421	1116
	10	ND*	38.76	26.4	657
3:5	7	3.32	30.82	193	2236
4:5	7	4.93	40.73	301	4291

\*ND = Not determined

**Table 2. The effect of wastewater dilutions on the levels of Mn, K and Na ( $\mu\text{g g}^{-1}$  d.w.) of *Ceratophyllum* at different incubation periods. Initial content in the control plant were 262  $\mu\text{g}$  Mn, 2497  $\mu\text{g}$  K and 1055  $\mu\text{g}$  Na.**

Wastewater Dilutions	Growth Periods (days)	Concentrations of essentials cations ( $\mu\text{g g}^{-1}$ d.w.) in <i>Ceratophyllum</i>		
		Mn	K	Na
1:5	9	509	2105	1387
	9	319	3487	2178
	10	14.50	2097	1045
2:5	9	497	2739	1920
	9	172	2125	1368
	10	ND	20048	3704
3:5	7	121	1285	1552
4:5	7	24	2331	1782

ND= Not determined