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Effect of pH and Temperature on the Uptake of Cadmium by *Lemna minor* L.

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Many aquatic macrophytes have the capacity to take up toxic heavy metals from polluted water and accumulate them (Chigbo et al. 1982; Aulio and Salin 1982; Charpentier et al. 1987). Cut leaves and intact plants have been suggested for clearing polluted water bodies of heavy metals (Salim 1988 a,b; Muramato and Oki 1983). However, uptake of metal ion from water is dependent on concentration, pH, temperature, presence of other substances and functional and morphological status of the biotic species (McLay 1976; Heaton et al. 1986). In an attempt to understand any correlation between metal bioconcentration, pH and temperature the optimal conditions for the removal of cadmium ions by duckweed, Lemna minor (L.) were studied.

MATERIALS AND METHODS

Lemna minor (L.)plants from axenic laboratory cultures maintained at the Industrial Toxicology Research Centre were used in the study. Modified Hoagland's medium (EPA 1975) was used throughout. Cultures were maintained under continuous fluorescent light (Philips) 200 μ mol m-2 s-1,at either 10, 20 or 30+0.5 °C. pH of the medium (pH 7.3 when freshly prepared) was adjusted to 6.5, 7.0 or 7.5 using a few drops of either 1 M citric acid or Tris solution. Only a very small amount of Tris was needed , thus chelation of metals would be negligible.

One hundred ml of culture medium was put into 250 ml flasks and calculated amounts of dilute cadmium chloride solution was added to give the final concentrations of 0.0 , 0.01, 0.1 and 0.5 ppm Cd

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(w/v). Four flasks were maintained at each concentration. The final volumes of all flasks were maintained at the same level by adding corresponding amounts of deionized water. The flasks were autoclaved at 15 lbs for 15 mins. Twenty fronds of the same size from laboratory stock cultures were put in each flask. Plants were harvested after 48 and 96 hours, washed with distilled water and a 20% homogenate (w/v) was prepared by homogenizing in a Potter- Elvehjem homogenizer in chilled , distilled water under cold conditions at medium speed for 2-3 minutes. Protein content of control and treated material was estimated by the method of Lowry et al. (1951) after removal of pigments from the trichloroacetic acid precipitate of the homogenate. Cadmium contents of whole homogenate, TCA precipitate and supernatants, which form protein and non protein fractions ,were estimated after digestion in 1N HNO_3 and 10 N H_2SO_4 using a Perkin -Elmer atomic absorption spectrophotometer model A.A.303. The student t ' test described by Fisher (1950) was employed to calculate the statistical significance values.

RESULTS AND DISCUSSION

The data for the uptake of Cd from the medium by Lemna as influenced by dose, time, pH and temperature are recorded in Table 1. The influence of temperature and pH on the uptake of Cd showed considerable quantitative variation in relation to amount of Cd and time. At 30°C, uptake was highest at pH 6.5 and Cd level of 0.5 ppm and the metal content showed 90% increase between 48 and 96 hrs ($p < 0.01$). The highest uptake at pH 7.5 was only about 60% that at pH 6.5 and at pH 7.0 the values were less than 15% . At temperature of 20°C the uptake pattern at 48 hrs was similar to that at 30 °C, with pH 7.0 being least effective. Plants grown at 10°C showed much less Cd content at pH 6.5 and 7.5 in all concentrations. However at pH 7.0 the Cd content was less affected. Thus the uptake and tissue content of Cd in Lemna varies with Cd exposure to time, pH and temperature.

Tables 2 and 3 indicate the data for Cd content of the protein and non-protein fractions from the plants maintained at 20 °C. The total protein content in the control and treated plants were similar. As such, the data are expressed in terms of fresh weight. As in the case of uptake, the distribution of Cd in the TCA insoluble and soluble fractions was also greatly influenced by pH and Cd concentration. At pH 7.5 the uptake increased with dose and time in both

Table 1: Influence of dose, time, temperature and pH on the uptake of Cd by Lemna minor (µg/gm dry wt.)

pH	Cd conc (ppm)	48 hr	96 hr
	Temperature **30 °C**		
6.5	0.01	3.73±0.29	5.60±0.61
	0.1	2.02±0.33	8.05±1.00*
	0.5	7.38±0.03	13.05±0.19***
7.0	0.01	0.03±0.03	1.83±0.11***
	0.1	Not done	Not done
	0.5	2.52±0.22	2.00±0.92
7.5	0.01	0.9±0.09	4.30±0.38**
	0.1	3.10±0.41	5.80±0.53
	0.5	4.81±0.53	7.70±0.87
	20 °C		
6.5	0.01	0.31±0.03	0.45±0.07
	0.1	3.15±0.41	4.22±0.50
	0.5	5.60±0.66	6.85±0.32
7.0	0.01	0.68±0.09	0.50±0.10
	0.1	0.12±0.02	0.60±0.09
	0.5	3.73±0.01	6.00±0.88
7.5	0.01	0.23±0.04	0.60±0.08
	0.1	4.15±0.04	5.00±0.67
	0.5	5.02±1.21	8.90±0.73
	10 °C		
6.5	0.01	0.02±0.00	0.10±0.02
	0.1	0.11±0.10	0.15±0.11
	0.5	0.09±0.10	0.15±0.04
7.0	0.01	0.10±0.07	0.94±0.00
	0.1	0.55±0.12	2.58±0.37
	0.5	3.80±0.30	4.27±0.28
7.5	0.01	0.16±0.04	0.90±0.07
	0.1	0.73±0.13	2.96±0.44
	0.5	1.00±0.21	1.58±0.26

Values are arithmetic mean ± SD of three replicates $p < 0.05$ *, $p < 0.02$ **, $p < 0.01$ ***, as compared with 48 hr samples

Table 2: Incorporation of Cd+2 in protein fraction of *Lemna minor* at 20 °C (ppm/gm tissue).

pH	Cd conc (ppm)	48 hr	96 hr
6.5	0.01	0.00±0.00	0.60±0.10
	0.1	0.06±0.02	0.76±0.04
	0.5	0.04±0.05	0.69±0.09
7.0	0.01	0.00±0.00	0.09±0.06
	0.1	0.30±0.04	0.20±0.05
	0.5	0.78±0.26	0.57±0.17
7.5	0.01	0.50±0.04	2.88±0.31
	0.1	0.86±0.04	3.19±0.04
	o.5	1.06±0.21	3.56±0.44

Table 3: Incorporation of Cd in non-protein fraction of *Lemna minor* at 20 °C (ppm/gm).

pH	Cd Conc (ppm)	48 hr	96 hr
6.5	0.01	0.00±0.00	0.08±0.02
	0.1	0.00±0.00	1.69±0.12
	0.5	0.64±0.01	6.85±0.77
7.0	0.01	0.00±0.00	0.28±0.01
	0.1	0.00±0.00	1.84±0.32
	0.5	4.30±0.31	4.78±0.44
7.5	0.01	0.00±0.00	0.28±0.07
	0.1	0.44±0.08	2.62±0.41
	0.5	3.51±0.05	3.56±0.29

fractions. In all cases the initial uptake was lower at the lower concentrations and showed an increasing trend with time and Cd concentration. At pH 7.0 the same pattern was also observed. However, the relative concentration figures showed that "protein bound" form was more in pH 7.5 , while protein-free form predominated at pH 7.0.The pattern was similar at pH 6.5 also. The profile of pH influence on Cd content in both fractions was similar in all concentrations and , generally the values also increased with concentration. Thus, maximum concentrations were found in TCA insoluble form at 96 hrs at pH 7.5 and 0.5 ppm Cd and the highest in TCA soluble fraction at 96 hrs at pH 6.5 and 0.5 ppm Cd.

The influence of time, dose,temperature and pH on the uptake of the metal from water could be through the physicochemical aspects of cation transport as well as the physiological and pathological status of the plant. The uptake was greater after the first 48 hrs, probably due to lapse in the initial ionic uptake which later was influenced by decreased metabolic activity as toxicity set in. It seems likely that plants growing at pH 7.5 are better able to form Cd-binding proteins as an effort to counteract pH stress. The chance of formation of Cd-inclusion bodies (Barcelo et al. 1988) could also be more at pH 7.5 so that they may be sedimented along with denatured proteins. At pH 6.5, where Cd could be in ionic form, the uptake was more enhanced by prolonged exposure than at the other two pH values. At pH 7.5 there was a preferential increase in protein-bound form and at pH 6.5 in the free form. It is apparent that as a result of pH stress the Cd uptake and sequestration mechanisms were altered. The decrease due to high pH was more prominent at 30 °C as compared to 20 °C,which could be due to higher solubility. The low concentration buildup in the tissue at 10 °C could be due to cold stress. At the optimal temperature of 20 °C, the effect of pH was less prominent. In fact ,at other conditions of temperature, pH or concentration, the biomagnification index was lower. Apparently, at the optimal conditions of plant growth there is better uptake and sequestration, till the pathmorphological lesions of toxicity develop.

Absorption of Cu and Cd cations by Lemna was supressed by chelators (Tanaka et al. 1982; Nasu et al. 1983) through functional changes in membrane structure and turger pressure altering availability of cations. Bioaccumulation and toxicity are influenced by pH (Greenham 1986). Membrane permeability is also affected by high and low temperature, thereby altering accumulation (Landolt 1957). The level of cadmium in the protein fraction was higher in plants growing at pH 7.5. The Cd in the TCA precipitate is indicative of sequestering mechanisms other than H-bond formation and could be taken as proteinaceous metal chelator rather than low molecular phytochelators. As toxicity sets in at higher concentrations of Cd, low uptake of metal is probably due to loss of membrane permeability to cations as the cell wall acts as a polyfunctional, weakly acidic, cation exchange facilitating exchange of metal cations (Stary and Kratzer 1984). Binding to cell surface may also cause alterations of the transport system . Increased influx of water or an increased permeability into the critical regions of

cells may explain resistance of certain species to heavy metal toxicity (Lustigman et al. 1985). The present results indicate that the effect of pH and temperature may be due to their influence on uptake per se and on physiological responses of the plant. The use of metal-tolerant plant families like Lemnaceae as abators of heavy metal pollution has been suggested (Hillman and Cully 1978). If such a fairly tolerant plant removes a substantial part of the biologically available toxic metal, it will help to reduce the stress on other more sensitive species of flora, fauna and microbiota. As this process is considerably influenced by temperature, pH and time of contact, optimal conditions for removal without affecting the propogation of the abator species could be experimentally worked out. The present data also indicate that the toxic effects and, therefore, regulatory safe limits of a toxic substance for aquatic species, could vary with environmental factors such as temperature and pH.

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