Bull. Environ. Contam. Toxicol. (1991) 47:764–769

Bulletin of Environmental Contamination and Toxicology

Chromium Uptake by *Spirodela polyrrhiza* (L.) Schleiden in Relation to Metal Chelators and pH

R. D. Tripathi and Prakash Chandra

Aquatic Botany Laboratory, National Botanical Research Institute, Lucknow—226 001, India

Duckweeds exhibit relatively high tolerance to heavy metal toxicity (Landolt and Kandeler, 1987) and are also capable of accumulating them (Nasu et al., 1983; 1985; Schreinmakers and Dorhout, 1985; Landolt and Kandeler, 1987). Because of these two characteristics, Lemna and Spirodela have been widely used for the removal of heavy metals from polluted waters (Landolt and Kandeler, 1987).

Among the heavy metals, chromium (Cr) is a major pollutant of water bodies as it is extensively used in tanning, and the effluent released during the processing causes severe Cr pollution in the water bodies (K.D. & G.K. pond) situated around the tanneries at Unnao (U.P.) India (Chandra, 1988; Garg and Chandra, 1990). These are regularly used for the cultivation of water chestnut (Trapa bispinosa), an important cash crop of the area. Surveys made recently have shown that a substantial amount of Cr is accumulated by the water chestnut (Chandra, 1988) and Cr at toxic levels enters into the food chain causing severe health hazards to local populations.

This paper reports the influence of metal chelators, ethylenediaminetetraacetic acid (EDTA) and salicylic acid, and pH on the accumulation of Cr by S. polyrrhiza under the laboratory conditions. This also includes the results of K.D. pond water treatment study by cultured fronds of S. polyrrhiza. In view of the occurrence of metal chelators in natural waters (Nasu et al., 1983; Dey, 1986; Fayed and Abd-El-Shafy, 1985) and pH variation (Chandra, 1988) the present study would enable to assess the performance of this species under the influence of these factors.

MATERIAL AND METHODS

Laboratory and field experiments were conducted to determine i) influence of metal chelators on Cr uptake, ii) regulation of

---

Send reprint request to R.D. Tripathi at above address.
NBRI Research Publication No.367 (N.S.)

Cr uptake by pH and iii) pond water treatment by cultured fronds.

Plants of S. polyrrhiza collected from an unpolluted water body were grown in 40% Hoagland nutrient solution (pH 6.2) under 16 h light (using fluorescent tube light, 114 μ mol m^{-2} s^{-1} at 25°+2°C) and 8 h dark period. New fronds produced in the culture were separated and used for the experimental work. In all experiments, pH was maintained by Tris buffer, 0.1 N HCl and 0.1 N NaOH.

The effect of metal chelators was determined by treating fronds (16) with six concentrations of Cr (0.025, 0.25, 2.5, 10, 18.1, 20.0 ppm) in 500 ml flasks. In each of these, the concentrations of EDTA (29.22 ppm) and salicylic acid (3.45 ppm) were kept constant (pH 6.2). The concentration of 18.1 ppm Cr was studied as it was found to be the LC-50 for S. polyrrhiza (Tripathi and Chandra, unpublished). In other experiments five different pH values (4,5,7,8,10) were maintained in each of these six concentrations of Cr.

The pond water (2 l) was treated with 10 g of cultured fronds in large plastic troughs (4 l capacity).

Three replicates of each concentration were used and the fronds in all the experiments were harvested after 2,3,7 and 14 days, dried and digested in HNO_3:$HClO_4$ (3:1,v/v). Water was digested using 5 ml HNO_3/100 ml pond water sample. Cr was estimated using Perkin Elmer 2380 Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

Substantial Cr uptake was detected in the treatments and increased with both external Cr concentrations and duration of the treatment. Maximum accumulation (1102 μg Cr g^{-1} dry wt.) was found at 20 ppm after 14 days but this was not significantly different from the value at 7 days.

Uptake of Cr was inhibited by EDTA. In the presence of EDTA, maximum accumulation was 992.0 μg g^{-1} dry wt. at 20 ppm Cr after 14 days. On the other hand the addition of salicylic acid in the medium increased the uptake substantially (Table 1).

pH regulated Cr uptake as shown in Table 2. In the acidic pH, uptake was stimulated whereas at basic pH (8 & 10) it was strongly inhibited. The maximum uptake (1138.00 μg g^{-1} dry wt.) was recorded at pH5 in 20 ppm Cr after 14 days. In contrast, the uptake was as low as 412.00 μg Cr g^{-1} (at pH 8) and 396.00 μg Cr g^{-1} dry wt. (at pH 10) for the same concentration duration.

Cultured fronds accumulated Cr from pond water (pH 6.75) having 0.25 ppm back ground Cr concentration. The accumulation increased progressively with increase in treatment duration,

Table 1. Cr accumulation ($\mu g\ g^{-1}$ dry wt) by S. polyrrhiza in the presence of EDTA and salicylic acid

Conc. (ppm)	CONTROL Days				EDTA Days				SALICYLIC ACID Days			
	2	3	7	14	2	3	7	14	2	3	7	14
0.025	100.0	146.2	200.0	202.0	92.0	136.0	183.0	185.0	239.7	346.3	366.2	402.0
0.25	200.0	289.7	346.2	362.5	173.2	200.0	279.7	342.0	299.7	579.4	692.6	706.0
2.5	526.2	590.7	705.0	872.0	233.0	346.5	486.2	512.0	406.2	759.2	1132.0	1245.0
10.0	592.7	699.3	800.0	892.0	432.9	679.3	685.0	765.9	1198.0	1485.0	1590.0	1590.0
18.1	672.9	894.0	1002.0	1052.0	639.3	692.6	945.0	962.0	1158.0	1605.0	1864.0	1872.0
20.0	745.9	922.0	1100.0	1102.0	705.9	792.5	979.0	992.0	1205.0	1684.0	1931.4	1945.0

Source of variation	df	SS	F Value
Days	3	1755444	101.85*
Chelators	2	4114262	358.08*
Conc.	5	8541800	296.63*
Chelators x conc.	10	928960	16.13*

* $P < 0.01$

Table 2. Effect of pH on Cr accumulation ($\mu g\ g^{-1}$ dry wt) by <u>S. polyrrhiza</u>

pH	Days	0.025 (ppm)	0.25 (ppm)	2.5 (ppm)	10.0 (ppm)	18.1 (ppm)	20.0 (ppm)
4	2	94.0	214.6	574.0	589.5	601.0	612.0
	3	111.0	251.0	609.0	652.0	676.5	701.6
	7	129.0	265.6	694.3	721.0	937.3	1032.6
	14	144.0	295.0	742.0	762.0	942.0	1035.0
5	2	99.6	235.6	666.0	712.3	803.0	824.0
	3	160.0	274.6	746.0	774.3	900.6	932.6
	7	205.2	358.0	763.3	807.3	1009.0	1131.6
	14	212.0	372.0	884.5	892.0	1092.0	1138.0
7	2	84.0	205.0	370.0	510.0	589.0	598.3
	3	101.0	215.0	420.0	513.2	610.0	693.0
	7	117.6	230.0	505.0	610.6	909.5	937.5
	14	147.0	280.0	594.5	702.0	932.0	944.0
8	2	41.0	56.0	105.0	207.6	298.0	307.6
	3	42.6	62.0	194.0	264.0	311.7	369.3
	7	74.0	109.0	237.0	305.0	309.0	407.0
	14	98.0	124.0	286.0	393.0	409.0	412.0
10	2	28.5	51.0	68.0	138.7	145.6	161.3
	3	42.6	53.6	113.6	157.7	179.2	192.0
	7	74.0	72.6	150.6	208.7	387.0	390.0
	14	90.0	102.0	172.0	288.0	392.0	396.0

Variation source	df	S.S.	F Ratio	Variation source	df	S.S.	F Ratio
Days	3	357498	18.07*	Conc.	5	5204248	157.91*
Treatment	29	10451206	54.67*	pH x conc.	20	1311364	9.947
pH	4	3938592	149.38*				

$^{*}P<0.01$

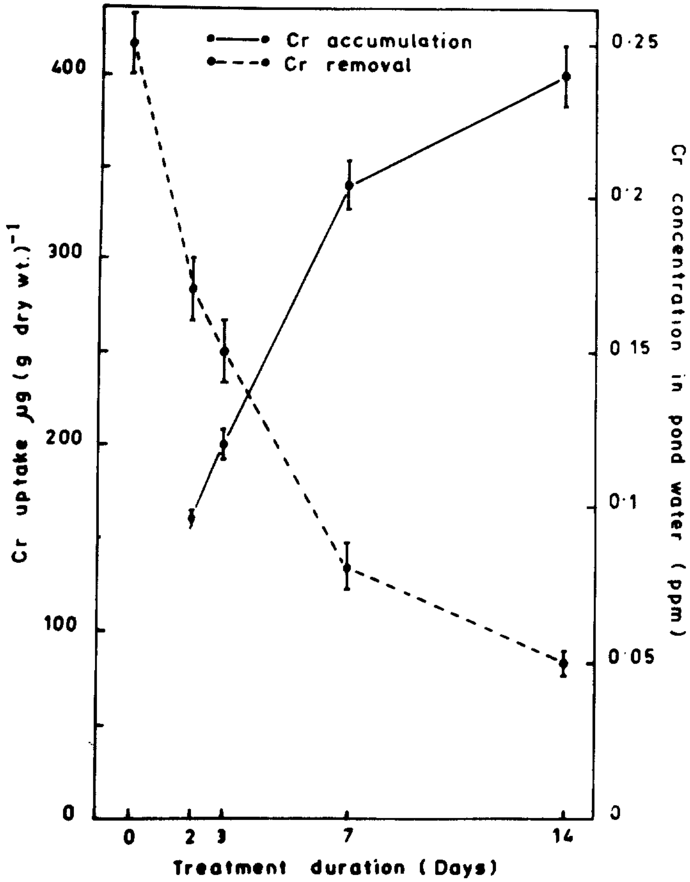

Figure 1. Chromium accumulation and removal from pond water (2 1) by Spirodela polyrrhiza (10 g). The error bars represent standard error of mean.

being maximum (400 ug Cr g^{-1} dry wt.) on 14th day. Cultured fronds demonstrated the ability of bring down the Cr concentration from 0.25 to 0.05 ppm in pond water during the treatment experiment (Fig. 1).

Staves and Knaus (1985) reported optimal accumulation of Cr by S. polyrrhiza at 10 ppm. However, in the present study the accumulation was less at the same concentration and approximately the same duration.

In Lemna spp. suppression of Cu and Cd accumulation by EDTA has been reported (Tanaka et al, 1982, Polar and KücüKcezzar, 1986). Fayed and Abd-El-Shafy (1985) made similar observation with respect to Cu, Zn, Cd and Pb. Our results with Cr conforms with these reports. Salicylic acid on the other hand has been reported to promote Cd and Cu uptake in Lemna paucicostata and

L. gibba (Tanaka *et al*, 1982; Polar and Kücükcezzar, 1986). In *S. polyrrhiza* similar observation was made by us. High Cr accumulation at acidic pH, as reported in water net alga (Rai and Chandra, 1989) was also found in the present study.

The results indicate that the fronds of *S. polyrrhiza* can accumulate Cr from polluted waters where metal chelators and variable pH conditions may exist. This knowledge can be useful while using this plant in reducing the level of Cr in closed water bodies.

Acknowledgment. Thanks to Dr. P.V. Sane, Director, National Botanical Research Institute, Lucknow for his interest in this work.

REFERENCES

Chandra P (1988) Identification of suitable plant species for biological water purification. Project Report on Technology Mission on Drinking Water, Nat Bot Res Instt pp. 44

De AK (1986) Environmental chemistry. Wiley Eastern Limited, Daryaganj, New Delhi-pp 265.

Fayed SE, Abd-El-Shafy HI (1985) Accumulation of Cu, Zn, Cd and Pb by aquatic macrophytes. Environ International 11: 77-87.

Garg P, Chandra P (1990) Toxicity and accumulation of chromium in *Ceratophyllum demersum*. Bull Env Contam Toxicol 44: 473-78

Landolt E, Kandeler R (1987) The family of Lemnaceae- a monographic study vol. 2. Veroff Geobot. ETH, Stftung Rubel, Zurich, 95 Heft.

Nasu Y, Kugimoto M, Tanaka O, Takimoto A (1983) Comparative studies on the absorption of Cd and Cu in *Lemna paucicostata*. Environ Poll 32: 201-209

Nasu Y, Kugimoto M, Tanaka O, Takimoto A (1985) *Lemna* as indicator of water pollution and absorption of heavy metals by *Lemna*. In: Pascoe D Edwards RW (Eds.) Fresh water biological monitoring, Pergamon Press, NY pp 113-120

Polar E, Kücükcezzar R (1986) Influence of some metal chelators and light regimes on bioaccumulation and toxicity of Cd^{+2} in the duckweed.(*Lemna gibba*). Physiol Plant 66: 87-93

Rai U N, Chandra P (1989) Removal of heavy metals from polluted waters by *Hydrodictyon reticulatum* (Linn.) Lagerheim. The Sci Total Environ 87/88 : 509-515

Schreinmakers WAC, Dorhout R (1985) Effect of copper ions on the growth and ion absorption by *Spirodela polyrrhiza* (L.) Schleiden. J Plant Physiol 121: 343-351

Staves RP, Knaus RM (1985) Chromium removal from water by three species of Duckweeds Aquatic Botany 23: 261-273

Tanaka O, Nasu Y, Takimoto A, Kugimoto M (1982) Absorption of copper by *Lemna* as influenced by some factors which nullify the copper effect on flowering and growth. Plant and Cell Physiol 23: 1291-1296

Received December 12, 1990; accepted May 22, 1991.