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Bioconcentration and phytotoxicity of chromium in *Eichhornia crassipes*

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Abstract: *Physico-chemical parameter and metal concentration in effluents of two industries i.e. Tannery industry, Jajmau, Kanpur and Electroplating industry, Scooter India Limited (SIL), Lucknow were determined to assess the toxicity of chromium. Metal accumulation in Eichhornia crassipes growing in these contaminated sites were also determined. For laboratory toxicity testing the plants were exposed to nutrient solution containing Cr concentration ranging from 0.01-10 $\mu g\ ml^{-1}$ for 24-96 hr. Accumulation of chromium was observed to be dependent on its concentration and time of exposure and was greater in roots (789.3 mg g^{-1} d.wt.) than in leaves (335.6 mg g^{-1} d.wt.) after 96 hr at 10 $\mu g\ ml^{-1}$ concentration. Under field conditions the accumulation of Cr was 1258 and 733.3 in roots and 94 and 53 $\mu g\ g^{-1}$ d.wt. in leaves of E. crassipes growing in Jajmau, Kanpur tanning industry and SIL effluents, respectively. It was found that lower doses (0.01-0.1 $\mu g\ ml^{-1}$) of chromium had stimulatory effect on various metabolic activities in plants including chlorophyll a, b and total chlorophyll, protein, nitrate reductase and mitotic index. Whereas higher doses of chromium had inhibitory effect. The carotenoid content and number of micronuclei was found directly proportional to the concentration of chromium and increased with increase in concentration of chromium to which plants were exposed. It may be concluded from the present study that E. crassipes is tolerant to the elevated Cr concentration as there is no inhibition of chlorophyll and carotenoid upto 0.1 $\mu g\ ml^{-1}$ at 24 and 48 hr exhibiting phytotoxicity at higher concentration. Therefore, E. crassipes may be used as bioassay for biomonitoring and control of Cr pollution in the environment.*

Key words: *Eichhornia crassipes, Chromium, Phytoremediation, Biomonitoring*

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Introduction

Chromium discharges from industries such as electroplating units, textiles, leather tanning and paper have increased its concentration several fold into surface water than its natural occurrence. Tannery effluent is a major source of aquatic Cr pollution in India with high BOD, COD and total dissolved solids. It occurs in several oxidation states ranging from Cr^{2+}, Cr^{3+} to Cr^{6+}. However, Cr^{6+} is the most toxic form and mutagenic (Kotas and Stasicka, 2000; Barnhart, 1997) because of its high solubility, ability to penetrate the cell membranes and strong oxidizing ability (Shanker and Pathmanabhan, 2004). Oxidative stress is caused either by inducing oxygen free radical production or by decreasing enzymatic and nonenzymatic antioxidants. Reactive oxygen species (ROS) reacts very rapidly with DNA, lipid and proteins causing cellular damage (Palma *et al*., 2002). Excessive Cr exposure causes skin ulceration, perforation of nasal septum, respiratory cancer, contact dermatitis, kidney damage and damage to various proteins, nucleic acid leading to mutation and carcinogenesis (Levis and Bianchi, 1982; Kiran *et al.,* 2008; Sahu *et al.,* 2007). IARC has determined that Cr is carcinogenic to humans (IARC, 1990). In plants it reduces root and shoot growth, chlorosis, stunting and finally plant death.

The toxic effect of Cr has amply been documented both in the laboratory and under natural conditions in aquatic plants (Sinha *et al*., 2002). Thus there is a great need to assess toxic potential of Cr in *E. crassipes* under such conditions. Wetland plant treatment is the best choice for treatment of wastewater because of the low maintenance cost and simplicity of operation (Ozengui and Elmaci *et al*., 2007). Recently, many plants including water hyacinth have become important in pollution treatment systems and used successfully to remove Cr. It will also be used well for genotoxicity testing due to well-developed profuse root system for monitoring and phytoremediation of low level of cadmium in water (Mishra *et al*., 2007).

The objective of present study was to assess bioconcentration, cytotoxicity, phytotoxicity and bioremediation potential of aquatic Cr by water hyacinth bioassay.

Materials and Methods

For physico-chemical analysis and Cr content, effluents were collected from industrial complex at Jajmau, Kanpur (leather industry) and Scooter India Limited (SIL), Lucknow, an electroplating industry. The physico-chemical properties of the effluent were determined using standard methods of APHA (2005). The plants of *Eichhornia crassipes* were also collected from these contaminated sites for determining Cr accumulation.

For laboratory experiment, plants of *E. crassipes* were collected from an unpolluted water body from Lucknow and acclimatized in hydroponic tubs under natural condition at Lucknow University, Lucknow. From this young plants were selected for experimental purposes. Cultures were placed in a growth chamber (light: dark cycle 14:10 hr, temperature 28±2°C, 115 $\mu mol\ m^{-2}s^{-1}$

illumination provided through day fluorescent tube light). Various concentration (0.0, 0.01, 0.1, 1.0, 2.5, 5.0 and 10.0 μg ml^{-1}) of Cr (VI) were prepared by adding the required aliquots of 1000 μg ml^{-1} stock solution of $K_2Cr_2O_7$ to the 5% Hoagland. The lowest experiment concentration of Cr was selected on the basis of permissible limit of Cr in the effluent (Srinathal *et al*., 2002)and the highest was that which could be toxic for the plants(Sinha *et al*., 2005). Plants of *E. crassipes* were transferred into 250 ml plastic beakers containing 200 ml chromium supplemented medium. Three sets, each of seven concentrations were placed separately in a growth chamber under conditions mentioned above. Plants placed in 5% Hoagland solution without chromium served as control. For the estimation of bioconcentration experimental cultures were aerated 6 hr a day. One set of each concentration was harvested after 24, 48, 72 and 96 hr of the treatment and washed three times with double distilled water. Oven dried (80°C) plant tissues (leaves and roots) were digested in HNO_3:$HClO_4$ (3:1 v/v) and chromium concentration was estimated by a flame atomic absorption spectrophotometer (Perkin Elmer 2380).

The chlorophyll and carotenoid contents of fresh leaves were estimated by the method of Arnon (1949) using 80% acetone. The chlorophyll and carotenoid concentration in mg g^{-1} of fresh leaves was calculated using the formula given by Duxbury and Yentsch (1956). Protein was estimated by the method of Lowry *et al*. (1951) using egg albumin as standard in roots or leaves. Nitrate reductase (NR) activity was assayed in leaves by the method of Srivastava (1974) and activity was expressed on fresh weight basis. Root meristems were fixed in Carnoy's fluid for mitotic index (Darlington and Lacour, 1976) and micronuclei end points (Panda *et al*., 1989).

The experiments were conducted taking three replicates (n=3) for each parameter. The data was subjected to test the significance of variation among the each parameter through two way ANOVA (Gomez and Gomez, 1984).

Table - 1: Physico-chemical characteristics of tannery and Scooter India Limited effluent

Parameters	Values	
	Tannery Jajmau, Kanpur	Scooter India Limited Lucknow
Colour	Light gray	Greyish green
Odour	Unpleasant	Unpleasant
Temperature (°C)	29.0±1.24	34.0±1.89
pH	7.8±0.62	7.9±0.63
DO	0.21±0.07	3.29±0.10
BOD	1349±6.01	370±3.62
COD	3791±18.48	1074±7.13
TS	3892±9.74	3754±8.04
TDS	3420±6.25	3315±7.59
TSS	472±3.79	439±2.34
Cr	1.305±0.012	0.782±0.013

Values are given in mg l^{-1} excepted pH, Temp., Odour, Colour. Mean ± SE (n=3)

Table - 2: Correlation coefficient (r^2) between metal accumulation in plant and metal content in water

Cr uptake	Contaminated site (Jajmau and SIL)	Laboratory concentration (1mg l^{-1} and 2.5 mg l^{-1})
Roots	1.002	1.002
Leaves	0.997	1.003

Results and Discussion

Physico-chemical analysis of effluent samples was carried out (Table 1). The effluents were slightly alkaline (pH 7.8 and 7.9) having dissolved oxygen (DO) 0.21 and 3.29 mg l^{-1}, biochemical oxygen demand (BOD) 1349 and 370 mg l^{-1}, chemical oxygen demand (COD) 3791 and 1074 mg l^{-1}, total solids 3892 and 3754 mg l^{-1}, total dissolved solids (TDS) 3420 and 3315 mg l^{-1}, total suspended solids (TSS) 472 and 439 mg l^{-1} and Cr content was 1.305 and 0.782 mg l^{-1} in Jajmau and SIL, respectively.

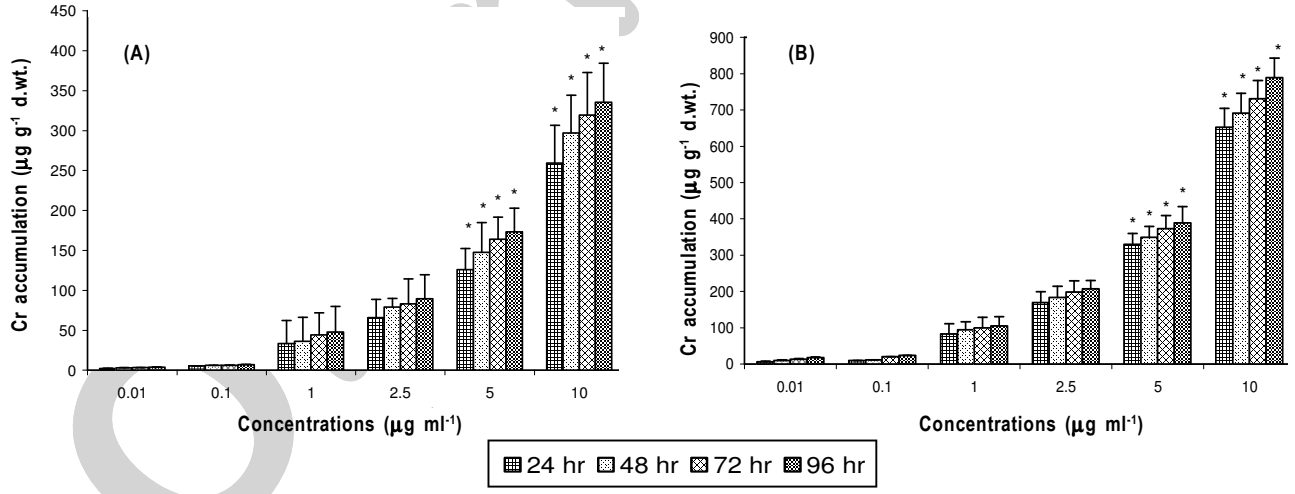

Fig. 1: Accumulation of chromium (μg g^{-1} d.wt.) in leaves (A) and roots (B) of *Eichhornia crassipes* at different concentration and duration (hr).

All values are mean triplicates ± S.D. * = Significance ($p<0.01$) compared to 0.01 μg ml^{-1} Cr

Table - 3: Effect of different concentration of chromium on photosynthetic pigments (mg g^{-1} fresh wt.) in *Eichhornia crassipes* at different exposures (hr)

Concentration ($\mu g\ ml^{-1}$)	Chlorophyll *a*				Chlorophyll *b*				Total chlorophyll				Carotenoid			
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
Control	1.27 ±0.09	1.30 ±0.07	1.35 ±0.07	1.36 ±0.08	0.38 ±0.04	0.41 ±0.06	0.42 ±0.05	0.44 ±0.06	1.66 ±0.06	1.72 ±0.06	1.75 ±0.05	1.80 ±0.08	0.34 ±0.08	0.36 ±0.08	0.39 ±0.04	0.40 ±0.08
0.01	1.31 ±0.07^{NS}	1.33 ±0.06	1.28 ±0.06	1.21 ±0.06	0.41 ±0.05	0.44 ±0.02	0.36 ±0.04	0.33 ±0.06*	1.71 ±0.08	1.76 ±0.05	1.66 ±0.05	1.52 ±0.04	0.41 ±0.06	0.43 ±0.05	0.46 ±0.06	0.51 ±0.09*
0.1	1.33 ±0.06*	1.36 ±0.06	1.25 ±0.05	1.15 ±0.08*	0.46 ±0.04	0.49 ±0.06	0.30 ±0.09*	0.27 ±0.09*	1.80 ±0.04	1.83 ±0.05	1.56 ±0.08	1.44 ±0.06*	0.42 ±0.06*	0.48 ±0.10*	0.55 ±0.05*	0.60 ±0.06*
1.0	1.26 ±0.05^{NS}	1.21 ±0.06	1.16 ±0.05*	1.11 ±0.06*	0.37 ±0.05	0.39 ±0.06	0.27 ±0.03*	0.21 ±0.04*	1.64 ±0.04	1.60 ±0.07	1.42 ±0.06*	1.30 ±0.07*	0.47 ±0.04*	0.52 ±0.10*	0.62 ±0.06*	0.69 ±0.06*
2.5	1.22 ±0.04^{NS}	1.17 ±0.05*	1.10 ±0.05*	1.03 ±0.05*	0.36 ±0.04	0.32 ±0.07	0.24 ±0.04*	0.19 ±0.05*	1.61 ±0.12	1.51 ±0.06*	1.31 ±0.08*	1.23 ±0.09*	0.52 ±0.05*	0.59 ±0.09*	0.69 ±0.04*	0.70 ±0.08*
5.0	1.18 ±0.05*	1.12 ±0.05*	1.07 ±0.05*	0.99 ±0.06*	0.30 ±0.07*	0.23 ±0.02*	0.20 ±0.10*	0.15 ±0.05*	1.50 ±0.06*	1.36 ±0.06*	1.26 ±0.05*	1.14 ±0.06*	0.54 ±0.06*	0.61 ±0.09*	0.58 ±0.06*	0.54 ±0.05*
10.0	1.12 ±0.05*	0.99 ±0.06*	0.88 ±0.08*	0.77 ±0.05*	0.28 ±0.07*	0.20 ±0.07*	0.18 ±0.07*	0.13 ±0.04*	1.39 ±0.04*	1.20 ±0.07*	1.04 ±0.04*	0.87 ±0.06*	0.60 ±0.10*	0.64 ±0.08*	0.61 ±0.04*	0.56 ±0.05*

All values are mean of triplicates ±S.D. * = Significance ($p<0.01$) compared to control

Table - 4: Effect of chromium on protein (mg g^{-1} fresh wt.) and nitrate reductase activity (µmol NO_2 g^{-1} fresh wt. hr^{-1}) in *Eichhomia crassipes* at different exposure (hr)

Concentration (µg ml^{-1})	Protein (mg g^{-1} fresh wt.)				NR activity (µmol NO_2 g^{-1} fresh wt. hr^{-1})			
	24	48	72	96	24	48	72	96
Control	91.56±0.86	92.46±0.53	91.62±1.1	91.33±1.0	84.23±0.14	85.14±0.20	87.37±0.21	88.10±0.07
0.01	91.77±0.85	92.55±0.72	92.84±0.78	92.25±1.2	89.19±0.19	79.0±0.15	71.22±0.10*	64.27±0.19*
0.1	91.84±0.98	92.61±0.62	93.14±0.59	92.48±0.75	81.37±0.10	76.29±1.2	67.03±0.62*	59.18±0.08*
1.0	89.67±0.62	89.32±0.98	84.23±0.85	81.56±0.72	78.05±0.81	65.16±1.76*	56.25±0.57*	53.28±0.50*
2.5	85.74±1.1*	82.68±1.1*	80.94±1.1*	78.18±0.89*	69.17±0.62*	57.08±0.23*	49.11±0.90*	41.07±0.13*
5.0	81.51±0.92*	76.17±0.80*	71.28±0.81*	68.49±0.52*	52.42±0.53*	48.21±2.14*	39.51±0.19*	30.42±0.06*
10.0	78.86±1.1*	70.51±0.56*	69.78±0.95*	64.27±0.87*	49.15±0.11*	41.37±1.88*	32.04±0.13*	25.21±0.13*

All values are mean of triplicates ± S.D. * = Significance ($p<0.01$) compared to control

Table - 5: Effect of chromium on mitotic index and number of micronuclei in *E. crassipes* at different exposure (hr)

Concentration (µg ml^{-1})	Mitotic index				Micronuclei			
	24	48	72	96	24	48	72	96
Control	7.51±0.05	7.65±0.08	7.49±0.09	7.46±0.07	1.42±0.08	1.43±0.15	1.41±0.08	1.38±0.14
0.01	7.63±0.06	7.75±0.06	7.33±0.08	7.18±0.05	4.30±0.16	5.25±0.17	6.18±0.16*	6.87±0.19
0.1	7.72±0.09	7.82±0.10	7.27±0.14	7.12±0.05	5.73±0.18*	6.35±0.16*	8.13±0.17*	10.22±0.13*
1.0	7.11±0.13	6.57±0.16	6.14±0.20	5.87±0.14	7.64±0.18*	8.14±0.15*	10.86±0.10*	13.43±0.18*
2.5	6.10±0.09*	5.44±0.19	5.10±0.19*	4.65±0.13*	9.27±0.16*	10.27±0.17*	13.65±0.19*	15.32±0.11*
5.0	5.35±0.20*	4.86±0.15*	4.22±0.15*	3.53±0.18*	10.14±0.25*	12.47±0.14*	15.53±0.15*	Toxic
10.0	4.14±0.20*	3.62±0.21*	2.24±0.09*	Toxic	11.72±0.14*	14.56±0.19*	18.34±0.15*	Toxic

All values are mean of triplicates ±S.D. * = Significance ($p<0.01$) compared to control

Physico-chemical analysis of effluents revealed alkaline nature and low DO levels. Jajmau effluent appeared to be more toxic than SIL since it had high BOD, COD and high Cr content. Penfound and Earle (1948) reported that rapid growth of water hyacinth takes place when the DO is 3.4-4.8 mg l^{-1}. Water hyacinth shows effective reduction in TS and BOD of sewage (Wolverton and Mc Donald 1979). It possesses tremendous potential to reduce the level of Cr, BOD and to improve other physico-chemical characters of wastewater.

In laboratory conditions, the accumulation of chromium was higher in roots than in leaves with increase in concentration of chromium and time of exposure (Fig. 1). Maximum amount of Cr was accumulated in roots of *E. crassipes* (789.3 µg g^{-1} dry wt.) followed by leaves (335.6 µg g^{-1} dry wt.) at 10 µg ml^{-1} Cr concentration after 96 hr of treatment (Fig. 1). Under field condition the accumulation of Cr was 1258 and 733.3 µg g^{-1} dry wt. in roots and 94 and 53 µg g^{-1} dry wt. in leaves of the plant growing in Jajmau and SIL effluents, respectively.

The translocation of Cr from root to shoot is restricted, as concentration of Cr in roots was higher than leaves in laboratory as well as in field condition. This is in agreement with earlier reports on Cr uptake by *E. crassipes* and other hydroponics (Jana, 1988). Such a high metal concentration in the root tissues may be due to immobilization of metal by cell wall and extracellular carbohydrates, which may be an important defense strategy, adopted by plants. Efficient binding and sequestration to the vacuoles by GSH (reduced glutathione) and PCs (phytochelatins) contributes to this high accumulation (Grill *et al.,* 1987). The lack of toxicity symptoms or any significant effect of Cr on plant growth appears to be the result of poor mobility as reported for other aquatic plants.

A strong positive correlation was found between Cr content in effluents and plant roots (r^2=1.002) and leaves (r^2=0.997). A similar trend was also found under laboratory condition (1.0 and 2.5 mg ml^{-1}) in roots (r^2=1.002) and leaves (r^2=1.003) (Table 2).

Chlorophyll *a, b* and total chlorophyll content increased at low concentration (0.01 and 0.1 µg ml^{-1}) and short duration (24 and 48 hr). However, there was significant decrease ($p<0.01$) at high concentration (1.0-10.0 µg ml^{-1}) and duration (72 and 96 hr) (Table 3). Maximum reduction was observed at 96 hr. Similarly, a concentration dependent reduction in chlorophyll content over control was also observed in the leaves of *Lycopersicon esculentum* (Gauba *et al*., 2007). However, carotenoid content showed dose dependent relationship. It increased significantly ($p<0.01$) with increase in concentration of Cr in the medium with duration (Table 3).

Heavy metal accumulation in vascular plant is known to produce significant physiological and biochemical responses (Jana, 1988). A decrease in chlorophyll content may either be due to inhibition of chlorophyll synthesis or its destruction or replacement of Mg ions (Barcelo *et al*., 1985; Chandra *et al*., 2009). An increase in carotenoid content was observed in Cr treated plants of *E. crassipes*. Increased carotenoid concentration for the protection from free radical formation is a common response to xenobiotics (Kenneth *et al*., 2000).

Table - 6: A summary table of first effect levels of different concentration of Cr to various parameters in *E. crassipes*

Parameters	Cr concentration exhibiting first effect (μg ml^{-1})	
	Stimulatory	Inhibitory
Cr accumulation in leaves	0.01	-
Cr accumulation in roots	0.01	-
Chlorophyll *a*	0.01	1.0
Chlorophyll *b*	0.01	1.0
Total chlorophyll	0.01	1.0
Carotenoid	0.01	-
Protein	0.01	1.0
NR activity	0.01	1.0
Mitotic index	0.01	1.0
Micronuclei	0.01	-

The effect of Cr on protein content is shown in Table 4. At lower concentrations Cr (0.01 μg ml^{-1}) had a stimulatory effect on protein content, showing maximum value of 93.14 mg g^{-1} fresh weight, but concentration above 0.1 μg ml^{-1} decreased the protein content ($p<0.01$) significantly.

Similarly, at lower Cr concentrations (0.01 μg ml^{-1}) and short duration (24 and 48 hr), *in vivo* NR enzyme activity was increased. On the other hand, higher Cr concentrations (0.1-10.0 μg ml^{-1}) at 72 and 96 hr exposure had significantly inhibitory effect (Table 4).

The decline in protein content under heavy metal stress in aquatic plants has been reported. A decrease in protein content in presence of heavy metal ions may be due to the breakdown of soluble protein or due to the increased activity of protease or other catabolic enzymes, which were activated and destroyed the protein molecules (Rai *et al*., 1998). However, the influence of Cr on NR activity seems to be because of reduced supply of NADH, which might result due to disorganization of the chloroplast, reduced rate of photosynthesis, respiration (Rees and Roberts, 1985). A positive correlation between NR activity and protein content has also been demonstrated in earlier studies (Rai *et al*., 1992; Vajpayee *et al*., 2000). The present findings are in agreement with earlier reports on Cr toxicity to chlorophyll, NR activity and protein content (Rai *et al*., 1992).

The mitotic index reflects the frequency of cell division and it is regarded as an important parameter to determine the rate of root growth. Mitotic index increased at lower (0.01-0.1 μg ml^{-1}) Cr concentration and exposure time (24 and 48 hr) while it decreased at higher concentration (1-10 μg ml^{-1}) and duration (72 and 96 hr). Maximum reduction (2.24) was found at 10.0 μg ml^{-1} at 72 hr exposure. The mitotic index can be correlated with the rate of root growth, suggesting that the inhibition of growth resulted from the inhibition of the cell division. Micronuclei cell frequency increased with increase in Cr concentration and duration. The concentration of 10 μg ml^{-1} was toxic at 96 hr exposure period ($p<0.05$) (Table 5).

In laboratory as well as *in situ* conditions, Cr inhibited cell division and altered the chromosomes (Gomez-Arroyo and Vilalobos-Pietrini, 1983). The presence of micronuclei indicates genetic damage induced by physical and chemical agents. MNC are induced through disturbance of spindle or through chromosome breakage (Yamamoto and Kikushi, 1980). Panda *et al*. (1989) suggested the use of MNC assay as a genotoxic end point. The present findings about mitotic index and micronuclei are in agreement with Pratap *et al.* (2006).

All these parameters depicted in Table 6 show that lower doses 0.01 and 0.1 μg ml^{-1} at 24 hr exposure had stimulatory effect on the activity of plants. On the other hand higher Cr concentrations had inhibitory effect. However, there was significant increase in carotenoid content and frequency of micronuclei with increase in dose.

The chromium removing potential of *E. crassipes* was quite promising as plant could accumulate significant amount of Cr in different plant parts under both field as well as from laboratory condition. It can be used safely at lower doses because showing stimulatory effect up to 0.1 μg ml^{-1} on physiological as well as biochemical parameters. *E. crassipes* can withstand wide range of pH, high BOD, COD, low dissolved oxygen and accumulate Cr from industrial effluent shows its practical utility. Therefore water hyacinth can be used as bioassay for biomonitoring and control of Cr pollution in the environment.

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