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Scavenging Potential of Hydrophytes for Copper Removal from Textile Dye Wastewaters

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With the growing urbanization and industrialization, the contamination of the water resources by heavy metals, is becoming a grave problem, the world over. The development of a sustainable technology for their removal is of paramount importance; as conventional methods of their removal are either very expensive or ineffective, especially when the metal concentrations are very low. In a large number of studies, the tendency of water plants to accumulate metals and other elements in excess of their physiological need has been demonstrated (Epstein 1972), even when they are present in smaller quantities (Wang et al. 1996). In the experimental studies, several aquatic plants have been found efficient in scavenging of heavy metals from synthetic wastewaters (Srivastav et al. 1994, Abdel Wahaab et al. 1995, Sajwan & Ornes 1996, Wang et al. 1996), but similar studies using industrial effluents are very few (Mishra et al. 1987, Sen & Mondal 1990).

Keeping this in view, the present study was made to select the most appropriate, locally available aquatic macrophytes for scavenging copper from textile dye wastewaters in Sanganer, Jaipur; where it is posing a major threat to both surface and ground water resources (Sharma et al. 2000).

MATERIALS AND METHODS

Acidic-azo and alkaline silicate dye wastewaters of the textile printing units in Sanganer were collected on alternate days during the study period. One young shoot (10-12cm long), each of *Cyperus, Phragmites, Polygonum* and *Typha* was planted separately in 10 L sized earthen pots, filled in with garden soil maintained under waterlogged condition, keeping 2-3 cm water above the soil surface. These were allowed to grow for 15 days. Thereafter, extra plants if any, were removed to maintain one plant/pot, after which plants of each species were divided into two groups; control plants and dye wastewater plants. The former was irrigated with tap water while the latter with dye wastewaters released during the third wash (dilute) of printed cloth, as the water of the first two washes (concentrate) was toxic to plants (Sharma et al. 1998). The dye wastewaters used during the experiments were of three types viz. acidic-azo (pH = 4.3-6.2, conductivity = 1.0-1.3 m mho/cm, COD = 223 - 470 ppm, Cu = 2.5 - 4.9 ppm), neutral-azo (cond-

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uctivity = 0.50-0.98 m mho/cm, COD = 150-280 ppm, Cu = 0.25-1.25 ppm) and alkaline silicate (pH = 8.0-9.0, conductivity = 1.47-1.7 m mho/cm, COD = 40-60 ppm, Cu = 0.1-0.2 ppm). The dye wastewater plants were again divided in three subgroups. The plants in subgroup A were irrigated with acidic-azo water, while of subgroup B with neutral- azo water, prepared by adding lime to acidic-azo water. The subgroup C plants were fed by silicate water containing reactive dyes. Two harvests were made at an interval of 30 and 45 days of plant growth in dye wastewaters. All plants of a pot were separated into shoot, standing dead, root and rhizome; whichever present. These were then dried in a hot air oven at 60°C, to constant weight; weighed and ground to a fine powder and finally digested in a mixture of HNO_3, H_2SO_4 and $HClO_4$ according to Piper (1957). Cu in the digested aliquots was estimated using Atomic Absorption Spectrophotometer (ECIL 4129). The tissue concentration of Cu was multiplied with the biomass of plant species to calculate the standing crop of Cu, in order to assess the potential of these macrophytes in striping off Cu from the dye wastewaters.

Unlike emergent macrophytes; the free-floating plant species *Azolla, Eichhornia, Lemna* and *Spirodela* were directly transferred to the 3L sized pots from their mono-cultures maintained in the Botanical garden, except for *Eichhornia*, which was grown in 10L sized pots. These plant species were grown separately in the tap water (control), acidic-azo, neutral-azo and silicate wastewaters. Unlike emergent macrophytes, two different experiments were set, namely azo and silicate. Two harvests were made at 7 days interval, in case of *Azolla, Lemna* and *Spirodela*; whereas for *Eichhornia,* these were four, made after 12,27,34 and 47 days of growth in azo wastewaters and at 14, 29, 36 and 41 days interval in silicate water. The harvested plants were dried and analyzed similar to emergent macrophytes.

5 cm long five ex-plants of *Hydrilla verticillata* and *Ceratophyllum demersum* were separately grown in 300 ml sized plastic pots, filled in with tap water (control), acidic-azo and neutral-azo wastewater. Two harvests were made at 7 days interval, and then processed for chemical analysis as described earlier. The data are based on three replicates in each set.

RESULTS AND DISCUSSION

In general, the growth of majority of aquatic macrophytes decreased markedly in the dye wastewaters as evident by their dry weights in comparison to the control plants at the termination of the study (Table1, 2). *Azolla, Ceratophyllum, Hydrilla, Lemna* and *Spirodela* died in acidic-azo water after their 7 days growth, which was delayed by one more week in *Polygonum*. Apart from these, *Cyperus* and *Eichhornia* also suffered severe retardation in their growth in acidic-azo water, while the latter was almost dead in silicate water. Unlike these, *Typha* growth in dye wastewaters was quite similar to control plants, whereas that of *Phragmites* was even better than control in the dye wastewaters. The adverse effects of dye wastewaters on aquatic macrophytes may be on account of their exposure to adverse pH of the medium (Akcin et al. 1993) including of higher concentration of Cu (Stoyanova and Tchakalova 1993, Abdel Wahaab et al. 1995).

The control plants had lower levels of Cu in comparison to those growing in dye wastewaters (Table 3,4 & 5); similar to *Elodea* grown in tap water devoid of copper sulfate (Stoyanova and Tchakalova 1993) which was added in the other set.

Table 1. Standing crops (g) of emergent and submerged hydrophytes growing in tap (control) and dye wastewaters at final harvest.

Plant species	Duration (Days)	Control	Neutral-azo	Acidic-azo	Silicate
Cyperus	45	44.4	18.8	11.6	39.7
Phragmites	45	35.9	53.1	37.9	45.7
Polygonum	45	11.3	3.9	Dead	Dead
Typha	45	50.5	26.2	10.0	27.0
*Ceratophyllum**	14	543	Dead	Dead	NA
*Hydrilla**	14	112	14.9	Dead	NA

*values in mg; NA = Not available

Table 2. Standing crops (mg) of free floating hydrophytes growing in tap (control) and dye wastewaters at final harvest.

Plant species	Growth (Days)	Azo-experiment			Silicate experiment	
		Control	Azo-neutral	Azo-acidic	Control	Silicate
Azolla	14	412	208	Dead	153	237
*Eichhornia**	47 (41)	8.4	4.1	3.2	8.7	2.1
Lemna	14	311	22	Dead	209	135
Spirodela	14	219	125	Dead	262	363

*value in g; Data in parenthesis related to duration of growth in silicate experiment.

The plants growing in acidic-azo water usually had maximum Cu concentrations, followed by those growing in neutral azo and silicate water (Table 3, 4 and 5). In *Ceratophyllum*, *Eichhornia* and *Hydrilla* however, the maximum Cu concentration was found in neutral-azo water.

The potential bio-availability of metals in sediments is favored by an increase in acidity, reducing power, salinity and concentration of either natural or synthetic ligands (Dunbabin and Bowmer 1992). In another study, Akcin et al.(1993) reported slower uptake of Zn in strongly basic solutions (pH=10) than the acidic solutions, similar to the present study. This may be due to the precipitation of Zn at pH higher than neutral, which decreased its availability for plant uptake; as reported by Wang et al.(1996) for Cu. This explains the higher uptake of Cu by the plants growing in acidic-azo wastewater where the Cu concentration is higher than both neutral-azo and silicate waters.
The tissue concentration of Cu in the aquatic macrophytes growing in dye wastewaters was in the order of; Submerged (2200- 5086 ppm) > Free floating (1344-3962ppm) > Emergent macrophytes (870-1855ppm).

Table 3. Tissue concentrations of copper (ppm) in emergent macrophytes after their 30 (I) and 45 (II) days growth in tap (control) and dye wastewaters.

Plant species		Control		Neutral-azo		Acidic-azo		Silicate	
		I	II	I	II	I	II	I	II
Cyperus	Sh.	47	13	135	187	870	810	73	67
	Rt.	35	28	229	563	657	545	85	167
	Std.	8	NA	448	493	529	726	102	83
St. crop (mg/m^2)		9.3 3.8*	28.7 8.0*	58.2 15.1*	131.9 30.8*	128.4 31.0*	152.6 29.8*	27.9 7.8*	17.2 36.9*
Phragmites	Sh.	10	46	31	31	146	135	10	17
	Rt.	10	50	149	300	755	294	36	107
	Rhz.	3	18	40	61	279	138	15	17
	Std.	NA	NA	NA	78	113	113	NA	16
St. crop (mg/m^2)		2.0 0.4*	28.5 10.1*	11.5 5.4*	75.2 51.6*	26.2 12.4*	111.2 39.4*	3.5 1.6*	48.4 42.0*
Polygonum	Sh.	18	13	24	21	Dead	Dead	20	Dead
	Rt.	91	71	2390	556	Dead	Dead	171	Dead
	Std.	NA	NA	19	36	437	204	NA	6
St. crop (mg/m^2)		2.2 0.8*	8.1 4.1*	17.2 15.4*	9.7 7.2*	Nil	Nil	2.8 1.1*	Nil
Typha	Sh.	8	13	34	36	195	45	9	13
	Rt.	29	48	114	263	1855	280	30	39
	Rhz	10	34	27	39	22	42	23	50
	Std.	5	21	205	342	397	235	35	20
St. crop (mg/m^2)		1.2 0.7*	23.4 15.6*	4.7 1.5*	43.7 17.5*	7.8 2.9*	70.8 31.9*	1.7 0.8*	14.8 11.4*

Sh. = shoot, Rt. = root, Std. = standing dead, Rhz. = rhizome; *standing crop of copper in shoot.

Table 4. Tissue concentration of copper (ppm) in *Azolla, Lemna* and *Spirodela* after their 7(I) and 14(II) days growth in tap (control) and dye wastewaters.

Plant species	Control		Neutral-azo		Acidic-azo		Silicate	
	I	II	I	II	I	II	I	II
Azolla	3	7	1373	1417	2128	Dead	441	390
St. crop (mg/m^2)	0.1	0.2	29.3	64	53	Nil	26.4	13.1
Lemna	4	2	800	NA	1344	Dead	98	78
St. crop (mg/m^2)	0.1	0.1	20.5	NA	24.6	Nil	3.0	2.8
Spirodela	14	36	836	1282	1625	Dead	177	186
St. crop (mg/m^2)	0.3	1.0	27.1	30.8	29.9	Nil	8.9	9.4
Ceratophyllum	30	NA	4556	Dead	Dead	Dead	NA	NA
St. crop (mg/m^3)	70.5	NA	455.5	Nil	Nil	Nil	NA	NA
Hydrilla	42	41	5086	4090	2200	Dead	NA	NA
St. crop (mg/m^3)	14.4	23	890	857	385	Nil	NA	NA

NA = not available, St. crop = standing crop

This difference in the behavior of different growth forms of aquatic macrophytes may be related to the proportion of absorption area available for the plant, in relation to the entire plant surface. The submerged hydrophytes, capable of absorption of minerals from their entire plant surface, therefore had maximum uptake of Cu, which perhaps decreased with the increase in proportion of the non- absorptive surface (shoot), especially in the emergent macrophytes.

Amongst free-floating plants growing in dye wastewaters, the tissue concentration of Cu was in the following sequence:
Eichhornia > *Spirodela* > *Azolla* > *Lemna*, whereas in emergent macrophytes the order was *Cyperus* > *Phragmites* > *Typha* > *Polygonum* (Tables 3,4 & 5).

Table 5. Tissue concentration of copper (ppm) in *Eichhornia crassipes* growing in tap (control) and dye wastewaters.

Treatment	Organ	I Harvest	II Harvest	III Harvest	IV Harvest
Control	Shoot	60	72	52	71
	Root	112	201	136	164
	St. dead	89	62	58	72
St. crop (mg/m^2)		3.5	9.1	7.9	17.6
Neutral-azo	Shoot	291	289	268	113
	Root	3962	3710	2750	1370
	St. dead	1680	2077	1730	1000
St. crop (mg/m^2)		57.1	90.4	92.4	50.4
Acidic-azo	Shoot	286	542	150	270
	Root	2269	2560	1360	1570
	St. dead	2010	2810	2480	1760
St. crop (mg/m^2)		42.6	63.4	64	58.8
Silicate	Shoot	169	224	173	155
	Root	500	1070	441	588
	St. dead	172	427	254	192
St. crop (mg/m^2)		13.9	28.9	14.8	13.5

St. dead = standing dead; St. crop = standing crop.

The levels of Cu absorbed by the tissues of different plant parts of emergent macrophytes were in the following order: Root > Standing dead > Rhizome ≥ Shoot.

The accumulation of heavy metals in the roots of majority of hydrophytes had been reported by several workers (Akcin et al.1993, Zaranyika and Ndapwadza 1995, Dushenko et al.1995, Mirka et al.1996, Ye et al.1998). This has been ascribed to their lower rate of translocation to shoots, and precipitation of most of the metals as sulfates (Reeves and Brooks 1983). In the present study also, the roots have been found to regulate the translocation of Cu to the shoot in all the four emergent macrophytes as well as *Eichhornia*, as evidenced by its higher levels in their roots as compared to shoot (Table 3, 5).

As, except for *Eichhornia*, the growth of *Azolla*, *Ceratophyllum*, *Hydrilla*, *Lemna* and *Spirodela* was adversely affected in dye wastewaters after their 7 days growth, and so, these plant species must be harvested after this period for copper scavenging. In the present study *Hydrilla* was found to be superior to *Ceratophyllum*, since it survived in dye wastewaters for a longer duration and also accumulated more Cu (385-890 mg/m^3) as compared to *Ceratophyllum* (455 mg/m^3). Contrary to the present study, *Ceratophyllum demersum* has been suggested as the most desirable plant species for scavenging heavy metals from wastewaters by Ornes and Sajwan (1993). Among free floating plant species, *Azolla* appeared to be the best for this purpose, as the amount of copper absorbed by it in 7 days (26-53 mg/m^2) is almost 2-3 folds higher than that absorbed by *Lemna* and *Spirodela* (3-30 mg/m^2) and almost equal to *Eichhornia* (14-57 mg/m^2) growing for 15 days in dye wastewaters (Tables 4,5). As optimum growth of *Azolla* is restricted to the winter season, the next plant species suitable for scavenging copper are *Eichhornia, Lemna* and *Spirodela* growing profusely throughout the year, especially the warmer periods.

In case of emergent macrophytes the standing crop of copper in the shoot has been considered for assessing their scavenging potential for copper. The maximum standing crop of copper, almost equal to *Azolla,* was recorded in the *Phragmites* shoots (39.4-51.6 mg/m^2) after their two months growth in the wastewaters (Table 3). The toxicity of wastewater to be treated, is however, the most crucial factor, to choose the plant species for scavenging, as it is very important that the plant species used, grows continuously in the wastewater for efficient and easy maintenance of the system. In view of this, we suggest the use relatively tolerant plant species identified during the present study for scavenging Cu from textile dye wastewaters. Among them, the submerged and free floating macrophytes may be considered for less toxic wastewater, while emergent macrophytes for the more toxic ones.

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