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Phytoremediation potential of aquatic macrophyte *Azolla caroliniana* with references to zinc plating effluent

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Abstract

The effect of zinc plating industrial effluent on some biochemical parameters and the phytoremedial capacity of floating aquatic macrophyte *Azolla caroliniana* was assessed. The effects of increasing zinc concentrations on growth characteristics of *A. caroliniana* namely root length, leaf area and biomass production was studied. The root growth of *A. caroliniana* was severely affected at higher concentration of zinc effluent (especially at 3 and 4%). Overall slight fluctuations in the moisture content represent the favorable nature of this essential micronutrient especially at 1% and 2% concentration. Maximum chlorophyll contents were observed at 2% concentration of the effluent with an increase by 41.29% over the control. The least amount of carotenoid was observed at 4% concentration. Polyphenol content of the fronds of *A. caroliniana* was lowest at 4% while higher accumulation was observed at 2% effluent concentration. The level of proline in the leaves of *A. caroliniana* was considerably increased under the influence of zinc especially at 1, 2 and 3% of the effluent concentration. It was also seen that electrical conductivity (EC) and total dissolved solids (TDS), content of the various dilution were decreased remarkably after 10 days of treatment. pH of the solution tend to become neutral while increase in turbidity might be related with root exudates and dead organics by the plant growing in the tray. The decrease in hardness, acidity, sodium and potassium content of the effluent were observed with increase in concentration of the effluent. Values for biological oxygen demand (BOD) were decreased in the effluent concentration of 2% while the decrease in chemical oxygen demand (COD) was maximum in the same effluent concentration after 10 days treatment period. Zinc was accumulated in increasing order in the plants treated with increasing concentration of zinc. The bio concentration factor (BCF) values were increased serially as per increasing concentration of zinc plating effluent and all the plants growing in these concentrations showed higher accumulation capacities. 4% of the effluent recorded with higher BCF while minimum for control treated biomass was observed. In the present study increasing BCF shows that the *A. caroliniana* has capacity to accumulate the Zn^{++} through liquid medium after ten days of exposure period.

Key words: *Azolla caroliniana*, Bioaccumulation, Biochemical and effluent parameters, Bioconcentration factor, Heavy metals, Phytoremediation

Introduction

Rapid industrialization and urbanization have resulted in elevated levels of heavy metals in the biosphere (Lu et al., 2004). Toxic levels of some heavy metals appear as a result of environmental pollution due to removal from mining, automobile traffic, smelting, manufacturing and agricultural wastes (Oncel et al., 2000). Over recent decades, the annual worldwide release of heavy metals reached 22,000 t (metric ton) for cadmium, 939,000 t for copper, 783,000 t for lead and 1,350,000 t for zinc (Singh et al., 2003). Chemical methods, to effectively decrease heavy metals to acceptable levels require a large excess of chemicals, which increase the costs because of generating the voluminous sludge (Spearot and Peck, 1984). Aquatic macrophytes play an important role in structural and functional aspects of aquatic ecosystems by various ways. The ability to take up heavy metals makes them interesting research candidates especially for the treatment of industrial effluents and sewage waters (Andra et al., 2010). Zinc compounds are widely used in industry to make paint, rubber, dyes, wood preservatives and

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ointments. Some amount of zinc is also released into the environment by natural processes, but most comes from human activities like mining, steel production, coal burning and waste burning (Andra et al., 2010). Zinc plays an important role in many biochemical reactions within the plants (Lu et al., 2004). Plants under stressful conditions adapt very differently from one another, even from a plant living in the same area (Mane et al., 2010). Phytotechnologies involving use of plants for pollutant removal is relatively a new approach and has gained importance during the last two decades (Dhir, 2010). The use of plants to degrade, assimilate, metabolize or detoxify contaminants is cost-effective and ecologically sound. Phytoremediation is rapidly gaining interest and promises in effective and inexpensive cleanup of hazardous waste sites contaminated with metals, hydrocarbons, pesticides and chlorinated solvents (Kumar and Chandra, 2004; Liao and Chang, 2004; Saratale et al., 2011). Several plants species, such as water lettuce (*Pistia stratiotes*) (Mishra et al., 2008), water lilies (*Nymphaea spontanea*) (Choo et al., 2006), parrot feather (*Myriophylhum aquaticum*), creeping primrose (*Ludwigina palustris*), and watermint (*Mentha aquatic*) (Kamal et al., 2004) have been studied to determine their potential in accumulating heavy metals.

Azolla caroliniana is a small aquatic fern which freely floats on the water surface but do not have roots embedded in sediment; it freely move with wind and water currents. It contains a symbiotic cyanobacterium-anabaena azolae within its leaf cavities. The *Azolla* macrophyte called frond ranges from 1.0-2.5 cm length in species *Azolla pinnata* and to 15 cm or largest, in *Azolla nitolica*. *Azolla* is available in Indian water bodies, where it is possible to use it in wastewater treatment by setting wastewater treatment ponds and by the way of ecofriendly technique. The N-fixing capacity of *Anabaena azolae* enables *Azolla* to thrive on nitrogen-free waters. It has been used as a fertilizer in botanical gardens because of nitrogen-fixing capability (Peters and Meeks, 1989). For several decades *Azolla* has been used as a green manure in rice fields. On the other hand, it has negative effects on the aquatic ecology due to its capable of colonizing rapidly to form dense mats over water surfaces (Ashton and Walmsley, 1976). *Azolla* has been shown to effectively adsorb hexavalent and trivalent chromium, zinc and nickel from solutions and electroplating effluent (Zhao et al., 1999) and gold from aqueous solution (Antunes et al., 2001). *Azolla* utilization in wastewater treatment to remove contaminants, particularly phosphorus, was also suggested by some authors (Fisher, 1988). *Azolla* is unique among floating macrophytes, because it can grow in waters devoid of combined nitrogen, due to the symbiosis with a N_2 fixing cyanobacterium, *Anabaena azollae* that lives in the dorsal lobe cavity of its leaf. So, this plant can grow even after the exhaustion of combined nitrogen in secondary effluents, improving an adequate phosphorus removal (Kitoh et al., 1993).

The present investigation demonstrates phytoremediation potential of *Azolla* exposed to zinc electroplating effluent. The effect was studied with reference to selected biochemical parameters and physico-chemical parameters of effluent. Growth characteristics, chlorophyll, carotenoid, polyphenol and proline content were studied so as to know the changes associated with the experimental species. Effluent quality analysis was carried out to assess the natural ability to treat the effluent. This was assessed with reference to electrical conductivity (EC), pH, total dissolved solids (TDS), total solids (TS), total suspended solids (TSS), turbidity, free CO_2, chemical oxygen demand (COD), biological oxygen demand (BOD), acidity, hardness, sodium and potassium content. Accumulation of zinc (Zn^{++}) was estimated to find out the extent of accumulation by *Azolla* from the effluent. Bioconcentration factor was also estimated to make out a clear conclusion about the phytoremediation potential of *Azolla* for the electroplating effluent at various concentrations.

Materials and methods

Materials

Zinc plating effluent was collected from MIDC Chikhli, Pune (20°19'17"N and 76°15'36"E). Fresh *Azolla* was collected from the surface of a water body near Damodar Nagar, Pune (18°28'28"N and 73°49'53"E) and cultured in a nutrient medium. Healthy mature plants (ten days old) were used for further study and estimation of biochemical and physico-chemical tests. Experiment was performed on a known weight basis in 500 ml plastic containers. As the effluent was much toxic and concentrated, lower dilutions were used for the experimental purpose. *Azolla* plants were exposed to tap water (control) and under elevated concentration of zinc plating effluent (1%, 2%, 3% and 4%). Plants were harvested after ten days of exposure for growth characteristics namely root length, leaf area, wet and dry biomass, moisture content, chlorophyll, carotenoid, polyphenol and proline content. The chemicals used in this study were of the highest purity available and of the analytical grade.

Estimation of photosynthetic pigments

Chlorophyll content

The chlorophylls of the mature fronds were estimated following the method suggested by Arnon et al. (1949) with slight modification. Fresh plant material (1g) was homogenized in mortar by keeping the temperature at 2°C in dark condition and extraction was carried out using 90% acetone, with the addition of pinch of magnesium carbonate, to protect and stabilize the chlorophylls. This extract was filtered through Whatman No.1 filter paper under suction using Buchner's funnel. Final volume of the filtrate was made to 100 ml with 90% acetone. Absorbance of the extract was recorded using double beam UV-Visible spectrophotometer (Elico SL-159, India) at 663 and 645 nm using 90% acetone as blank. Following formulae were used to determine the chlorophyll content.

Chlorophyll 'a' = X = 12.7 x A663 - 2.69 x A645
Chlorophyll 'b' = Y = 22.9 x A645 - 4.68 x A663
Total chlorophyll (a + b) =Z=8.02xA663+ 20.20 x A645

$$\text{Chlorophyll a / b/ total (mg/ 100 g)} = \frac{\text{X/ Y/ Z x volume of extract x 100}}{\text{1000 x weight of plant material (g)}}$$

Carotenoid content

The carotenoid content of the *A. caroliniana* fronds was determined from the same extract used for chlorophyll estimation, by recording the absorbance at 480 nm. The carotenoids content were calculated by using the following formula (Kirk and Allen, 1965).

$$\text{Carotenoids content (mg/100g)} = \frac{\text{A480 x volume of extract x 10 x 100}}{\text{2500 x weight of plant material (g)}}$$

Polyphenol content

The polyphenol content of the treated *A. caroliniana* fronds was estimated following the method suggested by Folin and Denis (1915). 2 ml of acetone extract used for chlorophyll was mixed with 10ml of 20% sodium carbonate and final volume was adjusted to 35 ml with distilled water, to this mixture 2 ml of Folin-Denis reagent was added, mixed thoroughly and final volume was made to 50 ml with distilled water. The standard tannic acid solution (0.1 mg ml^{-1}) was used for the preparation of standard polyphenol curve by measuring the absorbance at 660 nm.

Proline content

Proline was estimated from the oven dried *A. caroliniana* fronds, following the method described by Bates et al. (1973). The plant material was homogenized in 10 ml, 3% sulfosalicylic acid and the extract was filtered through Whatman No.1 filter paper. For assay, 0.5 ml of the filtrate was mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin reagent. The contents were boiled for 1 h. After cooling, 4 ml of toluene was added to test tube with vigorous shaking and the absorbance of toluene chromophore was recorded at 520 nm against toluene as blank. Standard curve of proline (0.1 mg ml^{-1}) was prepared by taking different concentrations of L-proline. From this standard curve, the proline content of different parts was calculated.

Growth characteristics

Ten fronds of *A. caroliniana* from each treatment tray were carefully removed, washed thoroughly with water to remove any dirt and dust particles and blotted to surface dry for the measurement of leaf area. Same material was used for the measurement of root length while completely grown material of each tray was removed carefully, blotted and used to estimate wet, dry biomass and moisture content.

Water quality analysis

Following parameters were determined from the diluted solutions of effluent taken out from plastic trays after 10 days. The methods used for analysis were in consistent with the standard methods mentioned in 'Handbook of Water Analysis' (Maiti, 2004).

Electrical conductivity (EC), total solids, total suspended solids and total dissolved solids (TS, TSS and TDS)

Electrical conductivity and total dissolved solids of the diluted effluent concentrations namely 1%, 2%, 3% and 4% were determined by using ELICO EC-TDS meter (CM 183, Make-India) where electrode was directly dipped into the respective solutions for the direct display of result on a digital scale. Total solids were determined by gravimetric method and then suspended solids were calculated by using equation $\mathrm{TS} = TDS + TSS$

Turbidity and pH

Turbidity of the sample was determined by using CL 52D ELICO Nephelometer while pH of the samples was recorded by using ELICO LI 127 pH meter.

Sodium (Na) and potassium (K) content

The sodium and potassium content of treated and untreated samples was determined with the help of Systronics-128 flame photometer. The air pressure was kept at 0.5 kg/cm² and the gas feeder knob was adjusted so as to obtain a blue sharp flame. Each sample was aspirated into a flame in the form of a fine spray under carefully controlled excitation. After recording readings a standard graph of absorbance vs concentration was plotted to estimate sodium and potassium contents.

Acidity

For determining acidity of the samples, 50 ml of content was mixed with 2-3 drops of methyl orange. In all the samples, methyl orange acidity was absent then the same content was titrated against 0.02N NaOH with the addition of 2-3 drops of phenolphthalein to obtain pink end point.

$$\text{Acidity (mg/L)} = \frac{\text{ml of 0.02 N NaOH x 1000}}{\text{ml of sample}}$$

Total hardness

The total hardness of the water samples was determined by EDTA titration method where 50 ml of well mixed sample was mixed with 1-2 ml buffer of pH 10 and a pinch of Eriochrome black-T indicator. The contents were then titrated with 0.01M EDTA till wine red solution changes to blue.

$$\text{Hardness (mg/L)} = \frac{\text{C x D x 1000}}{\text{ml of Sample}}$$

Where C=ml of EDTA for titration, D= mg of $CaCO_3$ equivalent to 1ml of EDTA

Chemical oxygen demand (COD)

COD determination was carried out with dichromate reflux method with the addition of 10 ml of 0.25N potassium dichromate ($K_2Cr_2O_7$) and 30 ml H_2SO_4+Ag_2SO_4 reagent in 20 ml diluted sample. The mixture was refluxed for 2 h and was cooled to room temperature. The solution was then diluted to 150 ml by using distilled water and excess $K_2Cr_2O_7$ remained was titrated with ferrous ammonium sulphate (FAS) using ferroin indicator.

$$\text{COD (mg/L)} = \frac{\text{(A - B) x N x 1000 x 8}}{\text{Volume of Sample}}$$

Where, A is the ml of FAS used for blank; B is the ml of FAS used for sample, N is the normality of FAS and 8 is milliequivalent weight of oxygen.

Biological oxygen demand (BOD)

The dilution method was followed to determine the BOD after three days at 27^{O}C. For the same dilution water was prepared with the addition of nutrients namely phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride. The diluted sample was transferred to BOD bottles of 300 ml capacity. After determining initial dissolved oxygen (DO) final DO was estimated from the bottles kept for incubation period for three days.

Zinc (Zn++) concentration

Zn^{++} concentration from the dried *A. caroliniana* plant material (frond and root combined). 1g oven dried plant material was acid digested as per the standard method suggested by Toth et al. (1948). Plant material was mixed with 10 ml concentrated nitric acid and then heated until all the material was completely dissolved. After cooling, 10 ml of perchloric acid was added and again heated until the solution became colorless and reduced to about 10%. After cooling, it was diluted to 100 ml with distilled water. Filtrate was used for analysis of Zn^{++} by using atomic absorption spectrophotometer (Perkin-Elmer, 3030A). Water samples from treated and untreated trays were directly used after filtration for the metal analysis.

Bioconcentration factor (BCF)

The bioconcentration factor provides an index of the ability of plant to accumulate metal with respect to metal concentration in substrate. The bioconcentration factor was calculated as the ratio of trace element concentration in plant tissues at harvest to the concentration of element in the external environment (Zayed et al., 1998). The BCF was calculated by using formula

$$\text{BCF} = P / E$$

Where, P represents the trace element concentration in plant tissues (ppm), E represents the residual concentration in water (ppm) or in sediment (ppm dry wt).

Percent removal efficiency

It was calculated by using following formula

$$Percent removal efficiency = \frac{\text{inlet pollutants - outlet pollutnats}}{\text{inlet pollutants}} x100$$

Statistical analysis

Statistical analysis of the data was carried out by using Graph Pad software. Mean standard deviation (SD) and percent variation was calculated. 'One Way Analysis of Variance' (ANOVA) was tested in order to check the statistical difference among the means. For the same Tukey-Kramer multiple comparison test of significance was carried out which suggested the variation among the column means is significant or not at different levels of significance. The data was analyzed for three different levels of significance based on the ‘p’ values as,

* Significant ($p = 0.01$ to 0.05),

** Very Significant ($p = 0.001$ to 0.01) and

*** Extremely Significant ($p < 0.001$)

Results and discussion

Physico-chemical analysis of zinc plating effluent was carried out with respect to selected parameters (Table 1). The concentration of zinc (Zn^{++}) was observed to be at higher levels as expected and was 751.65 mg/l. The effluent was acidic with higher electrical conductivity, in addition with increased levels of TDS, TSS and in turn total solids. Sodium and potassium content of the samples were also much higher and might be due to the salts used in the various processing units for the electroplating. In general, the chemical constituents and dissolved contents were higher and are directly responsible for increase in COD and BOD values of the untreated effluent.

Table 1. Characteristics of untreated zinc plating industrial effluent.

Sr. No.	Parameter	Value
1)	pH	3.11 (±0.11)
2)	EC	7740.12 (±12.73)
3)	TDS	4330.34 (±10.64)
4)	TSS	320.45 (±8.25)
5)	TS	4650.79 (±14.41)
6)	Turbidity	9.9 (±0.31)
7)	Hardness	1250.45 (±5.61)
8)	Acidity	1680.71 (±6.38)
9)	Na	550.51 (±6.13)
10)	K	240.5.56 (±11.42)
11)	Zn	751.65 (±4.82)
12)	Fe	22.32 (±1.42)
13)	COD	1164.23 (±16.57)
14)	BOD	232.41 (±13.45)

Values are mean of three observations with ±SD in parenthesis, expressed in mg/l except pH unitless, EC in µS and turbidity in NTU.

Growth analysis

Growth changes are often first and most obvious reactions of plants under heavy metal stress (Hagemeyer, 1999). In present study plant growth was measured in terms of root length, frond area and biomass production in terms of fresh and dry weight. The analysis of growth characteristics indicates that *Azolla* can grow quite well in treated urban wastewater, having growth rates and productivities great enough to encourage its utilization as biofilter for nutrient removal and biomass production (Zhao et al., 1999). Alicia et al. (1994) reported that roots of some aquatic plants could retain both coarse and fine particulate organic materials present in water bodies supporting their growth.

Root length

Maximum root length was observed the biomass grown at 2% of the effluent with 4.5 cm while minimum was observed as 1.9 cm in the tray supplied with 4% zinc plating effluent (Table 2). The root length was also observed to be decreased with the increase in concentration of zinc in the sets provided with 2, 3 and 4% of the effluent which directly indicates the negative effect of such higher concentration of zinc present in hydroponics. Schat et al. (1996) had reported that Zn toxicity in plants was associated with reduced root growth of fronds. It has been reported that the non-lethal concentration of Zn^{++} shows an inhibitory effect on cell division in root tips of *Nigella sativa* and *Triticum aestivum* (El-Ghamery et al. 2003). Literature survey showed that the heavy metals accumulations in water hyacinth increased linearly with the solution concentration in the order of leaves<stems<roots (Stratford et al. 1984). Munns (2003) concluded that the reduction might be attributed to the inhibition of hydrolysis of reserved foods and their translocation to the growing shoots. From the present investigation, it is clear that the root growth of *A. caroliniana* was sensitive and severely affected at higher concentration of zinc (especially at 3 and 4%) which might be due to higher uptake of $Zn^{++}$and harmful nature of effluent.

Table 2. Effect of zinc plating effluent on selected growth parameters of *A. caroliniana* (after 10 days treatment).

Parameter	Zinc plating effluent concentration %				
	Control	1	2	3	4
Root length (cm)	4.3 (0.19) 0.0[a]	4.5* (±0.25) 4.65	4.1*** (±0.29) -4.65	2.4** (±0.15) -44.19	1.9(±0.34) -55.81
Frond area (cm^2)	4.5(0.11) 0.0 [a]	4.7(±0.45) 4.44	4.4*(±0.34) -2.22	3.7***(±0.24) -17.78	3.2*** (±0.09) -28.89
Fresh weigh (g)	4.040.0 [a]	4.99**23.51	5.65*39.85	4.09*** 1.24	3.85*** -4.70
Dry weigh (g)	1.320.0 [a]	1.35*2.27	1.5114.39	1.54** 16.67	1.64* 24.24
Moisture content (%)	67.40.0 [a]	68.57***1.74	73.2**8.61	62.32* -7.54	59.90*** -11.13

a The values indicates percentage variation relative to initial values. Values are mean with ±SD. Significantly different from the control at *P<0.05, **P<0.01 ***P< 0.001 by one-way ANOVA with Tukey-Kramer multiple comparisons test.

Frond area

It is evident from the results that the frond area was 4.7 cm² in 1% zinc effluent and was maximum while minimum was observed at 4% effluent by a value of 3.2 cm² 10th day of experiment. It was slightly decreased at 2% of effluent in the potting medium. Average frond area also showed decreasing trend with the increasing concentrations of zinc in the growing medium similar to root length. The mechanism of plants behind for growth reduction is not clear to maintain residual growth for short and long term responses (Munns, 2003). The growing grass leaf represents an inherent combination of growth, developmental and micro-environmental zones and this combination can be used to identify signaling events, which are involved in the regulation of growth and growth responses to stress (Fricke et al., 2006). From the present results it is clear that frond area affects at higher percent of effluent and might be associated with higher levels of zinc in the potting medium.

Biomass

Fresh weight

It is well known that aquatic biomass irrespective living or dead, exhibits capacity to remove heavy metals from wastewater. In the present study, maximum fresh weight as 5.65g was recorded in 2% effluent concentration while minimum fresh weight as 4.11g in the tray supplied with 4% concentration of zinc plating effluent (Table 2). The increase in the fresh weight of the plants at lower concentrations i.e. at 1% and 2% of Zn plating industrial effluent might be due to favorable nature of this essential element in plant growth. The reduction in biomass production is associated with chlorosis and necrosis of the leaves that reduce the photosynthetically active area (DeHerralde et al., 1998).

Dry weight

Maximum dry weight was observed at 4% concentration when whole biomass was weighed after drying. It was 1.64g while minimum dry weight was 1.32g in control tray. Increase in dry weight of the experimental species showed perfect positive correlation with the increase in the concentration of the effluent. Jarunee et al. (2003) observed the inhibition in dry weight of shoots and roots in *Sesbania rostrata* while Jain et al. (1990) found that the addition of Zn^{++} at low concentration had a favorable effect on growth of water hyacinth, which may be attributed to the favorable nature of Zn^{++} as micronutrient for the growth. In the present study an increase in dry weight of *A. caroliniana* might be due to accumulation of zinc and its favour by the azolla biomass as a micronutrient.

Moisture content

It is seen from the Table 2 that the moisture content was not much affected and increased at 1 and 2% concentration of effluent. There was slight decrease with the increased concentration later on. Maximum moisture content was observed by 73.20% in 2% of the effluent at the end of the treatment whereas minimum moisture content was 59.90% at 4% effluent in the tray. The decrease in the moisture content at 4% concentration shows that prolonged and concentrated exposure had a negative effect on the biomass growth. Overall slight fluctuations in the moisture content represent the favorable nature of this essential micronutrient especially at 1% and 2% concentration.

Table 3. Effect of zinc on chlorophyll content of *A. caroliniana*.

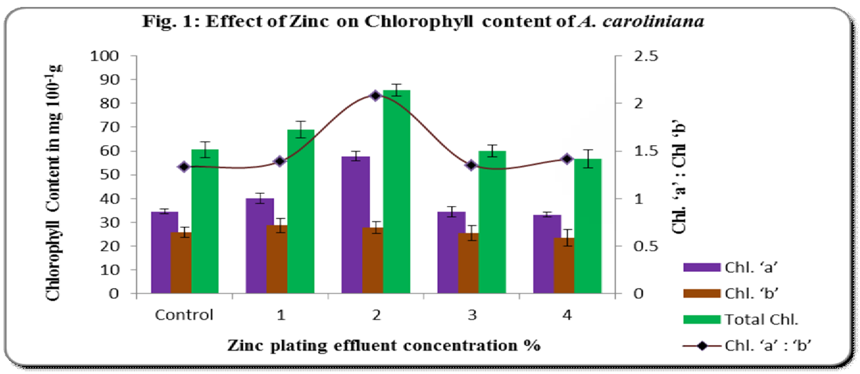
Chlorophyll	Zinc plating effluent concentration %				
Content	Control	1	2	3	4
Chl. 'a'	34.56 (±1.02) 0.0[a]	40.14**(±2.21) 16.15	57.72***(±1.98) 67.01	34.50***(±2.15) -0.17	33.27*(±0.95) -3.73
Chl. 'b'	25.98(±2.15) 0.0[a]	28.8*(±3.01) 10.85	27.87**(±2.57) 7.07	25.5***(±3.29) -1.85	23.52***(±3.51) -9.47
Total Chl.	60.54(±3.24) 0.0[a]	68.94***(±3.42) 13.88	85.54***(±2.58) 41.29	60.0***(±2.49) -0.89	56.79***(±3.81) -6.19
Chl. 'a' : Chl 'b'	1.33**(±0.029) 0.0[a]	1.39**(±0.024) 4.77	2.08*(±0.21) 55.99	1.35***(±0.032) 1.71	1.41***(±0.035) 6.34

a The values indicates percentage variation relative to initial values. Values are mean of three experiments and are expressed in mg 100-1g of fresh tissue with ±SD. Significantly different from the control at *P<0.05, **P<0.01 ***P< 0.001 by one-way ANOVA with Tukey-Kramer multiple comparisons test.

Biochemical parameters

Effect of zinc on chlorophyll content of *A. caroliniana*

The effect of zinc samples on chlorophyll content of mature fronds of *A. caroliniana* is shown in Table 3 and Figure 1. It is evident from the results that the total chlorophyll content of the mature fronds was increased considerably due to increasing concentrations upto 2% of zinc plating effluent but later on decreased with further increase in concentrations. The decrease in chlorophyll contents at 3 and 4% was not remarkable; it was slight as compared to the control set. Maximum chlorophyll content was 85.54 mg 100^{-1}g was recorded at 2% effluent concentration while minimum chlorophyll content was observed at 4% concentration by a value of 56.79 mg 100^{-1}g. In general chlorophyll 'a' appears to be dominant over chlorophyll 'b', this fact was observed to be true even in case of elevated levels of zinc plating effluent. The ratio was not much affected and was increased markedly by a value of 2.08 at 2% concentration of the effluent which shows the favorable nature of the zinc present in the hydroponics. Thus, it is quite clear that the higher salt concentration (4%) is not much negatively influential on 'a': 'b' ratio. Several cases of decreased chlorophyll content owing to metal toxicity have been reported in the plant kingdom growing in wetland ecosystems (Valavanidis et al., 2005). Chlorophyll degradation is the routinely observed response to stress or chiefly in elevated concentrations of various heavy metals (Chen and Djuric, 2001). Our results on chlorophyll pigments are in accordance with the findings of Mishra et al. (2007). Similarly, concentration dependent reduction in chlorophyll content over control was also observed in the leaves of *Lycopersicon esculentum* (Gauba et al., 2007). The slight decrease in chlorophyll content at 3 and 4% effluent in *A. caroliniana* might be due to accumulation of Zn^{++} and other toxic effluent constituents. The increase in total chlorophyll content in fronds of *A. caroliniana* might be due to osmotic adjustment mechanism developed by experimental species. The total chlorophyll content decreased as the concentration further increased from 2% to 4%.



Effect of zinc on carotenoid content of *A. caroliniana*

According to Armstrong (1996) carotenoids have two major functions in photosynthesis, they protect chloroplast from photo-oxidative damage and they also act as accessory light harvesting pigments because they absorb the light energy in the range of 400-500 nm (blue), which is not accessible by the chlorophylls and pass this excitation energy to chlorophyll molecules. They are also one of the non-enzymatic antioxidants along with vitamin C, vitamin E and lipoic acid and play an important role in the protection against oxidative stress (Kojo, 2004). Kenneth et al. (2000) reported that an increase in carotenoid content was observed in Cr treated plants of *E. crassipes* and such an increase for the protection from free radical formation is a common response to xenobiotics. From the results it is clear that the carotenoid content of the fronds of *A. caroliniana* showed a decreasing trend but it was not much remarkable and was directly proportional to the increase in the concentration of effluent in the liquid medium (Table 4 and Fig. 2). Chen and Djuric (2001) were of the opinion that decrease in carotenoid content is a regular response to stress condition due to heavy metals. Maximum amount of carotenoid content was observed with a value of 25.2 mg 100^{-1}g in the fronds grown in control pot while least carotenoid content was observed at 4% concentration with 21.28 mg 100^{-1}g. It is clear that total carotenoid content of the fronds of *A. caroliniana* was reduced due to the increasing zinc concentration in the pots and proved to be a negative photo-oxidative impact while a slight increase at 4% but below the control, might to resist the toxic nature effluent.

Effect of zinc stress on levels of polyphenol content of *A. caroliniana*

Very little attention has been paid towards the influence of metals on the polyphenol metabolism in plants. Parida et al. (2002) were of the opinion that accumulation of polyphenols played a key role in plants towards stress. In the present study, increased levels of polyphenols especially at 1 and 2% of effluent might have induced accumulation of secondary metabolites in the experimental species to tolerate the stress condition while decrease at 4% level might be due to damage in polyphenol production process. Polyphenol content of the fronds of *A. caroliniana* was lowest by 144.27 mg 100^{-1} g at 4% while higher accumulation was observed at 2% effluent concentration by a value of 334.89 mg 100^{-1}g (Table 4 and Figure 2). Increase in polyphenols of the fronds of *A. caroliniana* at all concentrations of the zinc plating effluent might be related to the modified tolerance mechanism adopted by the plants for overall growth and development.

Effect of zinc stress on levels of proline content of *A. caroliniana*

Proline occurs widely in higher plants and accumulates in larger amounts than other amino acids, regulates the accumulation of usable nitrogen (Abraham et al., 2003). Proline accumulation is considered to be involved in stress resistance mechanisms (Lutts et al., 1999). It is clear from the results (Table 4 and Fig. 2) that the level of proline in the leaves of *A. caroliniana* was considerably increased under the influence of zinc especially at 1, 2 and 3% of the electroplating effluent. Maximum increase (79.06%) in proline content was observed by 14.54 mg 100^{-1} g at 1% of the effluent concentration and the minimum (-12.32%) proline content was observed by a value of 7.12 mg 100^{-1} g at 4% of effluent concentration. It is evident that in general proline accumulates in plants exposed to various environmental stresses and is not an exception to toxicity stress of zinc plating effluent at various concentrations. The increased levels of proline in the leaves of *A. caroliniana* might be able to maintain osmoregulation and conservation of energy for a stress developed due to zinc containing effluent while decrease at 4% concentration indicates the toxicity of the effluent.

Table 4. Effect of zinc plating effluent on carotenoid, polyphenol and proline content of *A. caroliniana*.

Parameter	Zinc plating effluent (%)				
	Control	1%	2%	3%	4%
Carotenoid	25.2(±2.58)	24.08*(±3.04)	23.44**(±3.61)	22.0***(±3.46)	21.28**(±1.59)
	0.0[a]	-4.44	-6.98	-12.70	-15.56
Polyphenols	156.24(±6.32)	334.89**(±11.45)	305.24*(±8.78)	164.78***(±4.92)	144.27*(±3.78)
	0.0[a]	114.34	95.37	5.47	-7.366
Proline	8.12(±1.48)	14.54**(±1.35)	9.64***(±0.98)	8.58*(±1.05)	7.12***(±0.86)
	0.0[a]	79.06	18.72	5.67	-12.32

a The values indicate percentage variation relative to initial values. Values are mean of three experiments and are expressed in mg 100-1g of fresh tissue with ±SD. Significantly different from the control at *P<0.05, **P<0.01 ***P< 0.001 by one-way ANOVA with Tukey-Kramer multiple comparisons test.

Effect on water quality parameters

Electrical conductivity (EC) and total dissolved solids (TDS)

It is evident from the Table 5 that the EC of the samples was decreased after 10 days of treatment in all trays. Maximum reduction was observed by in control set (-18.34) followed by 2% of effluent concentration (-16.65). This clearly shows the capacity of *A. caroliniana* to treat the effluent. Mahmood et al. (2005) also observed the reduction conductivity due to absorption of pollutants by plants. TDS content of the samples was also decreased after 10 days treatment period where it was maximally reduced in control set followed by 2% and 3% of the effluent. Even in 4% of the effluent the TDS content was reduced by 29.68%. Khosravi et al. (2005) reported the importance of TDS uptake by *Azolla filiculoides* for their growth in wetlands. Groudev et al. (2011) observed reduction of total dissolved solids from 2620 ppm to 1230 ppm in treatment of acid mine drainage from an uranium deposit by means of a natural wetland. The reduction of TS was due to the retaining coarse and fine particulate organic materials present in water bodies supporting their growth by the root system while TSS content was reduced in all the cases after the retention time of 15 days (Ghaly, 2004). A good reduction (90%) of total suspended solids by constructed wetland plants with a retention time of 7 days was reported (Amelia, 2001).

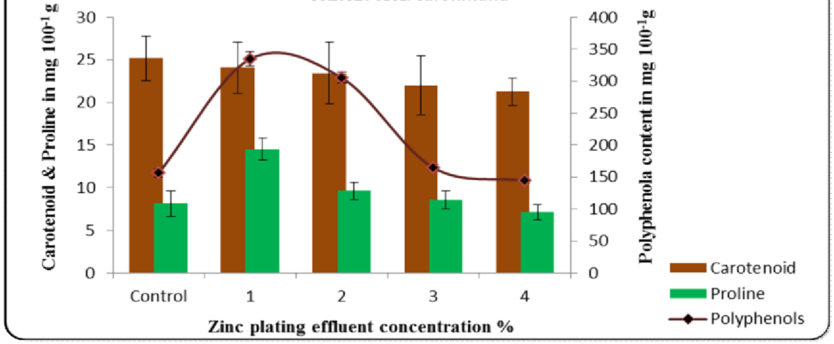


Fig. 2: Effect of zinc plating effluent on carotenoid, polyphenol and proline content of *A. caroliniana*

pH and turbidity

It is evident from the Table 5 that the pH of samples decreased with the increase concentration of effluent initially on day zero but when compared to final values with respective percentage of the solutions the values tend towards higher pH. The minimum pH was observed as 4.45 at 4% concentration on zero day while maximum pH as 5.91 was observed in the same tray after 10 days of treatment with *Azolla*. It clearly indicates that the experimental species try to grow and maintain the pH towards neutral which might be due to the root secretions produced to avoid negative effect of toxins. Optimum growth of *Azolla* in culture solution is in pH range of 4.5-7, but *Azolla* can survive in pH 3.5-10 if all essential elements are available (Ashton and Walmsley, 1974). Wagner (1997) found that at water temperature of 25 ^{o}C, both *A. pinnata* and *A. filiculoides* showed maximum growth at pH values from 5-7. Watanabe (1984) reported that *Azolla* growth was decreased by increasing pH level at low iron concentration. pH seems to be the most important parameter in the biosorptive process: it affects the solution chemistry of the metals, the activity of functional groups in biomass and competition of metallic ions (Galun, 1987). The pH was reduced from alkaline to nearly neutral by treatment with aquatic macrophytes (Mahmood et al., 2005).

Table 5. Reduction in electrical conductivity (EC), total dissolved solids (TDS), pH and turbidity of zinc plating effluent grown with *A. caroliniana* after 10 days.

Zinc plating effluent(%)	EC (µS)		TDS (mg/l)		pH		Turbidity (NTU)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control	299.21 (±2.62) 0.0[a]	244.34 (±2.61) -18.34	149.59 (±1.62) 0.0[a]	89.47 (±0.56) -40.19	5.25 (±0.21) 0.0[a]	5.29 (±0.15) 0.76	0.18 (±0.08) 0.0[a]	1.23 (±0.02) 583.33
1	356.52* (±3.25) 0.0[a]	312.64*** (±3.12) -12.31	179.58** (±1.48) 0.0[a]	119.61* (±0.48) -33.39	5.17** (±0.15) 0.0[a]	5.37*** (±0.19) 3.87	1.3* (±0.07) 0.0[a]	6.26 (±0.03) 381.54
2	416.86** (±2.65) 0.0[a]	347.45* (±3.32) -16.65	207.61 (±2.12) 0.0[a]	137.48** (±0.98) -33.78	5.08** (±0.24) 0.0[a]	5.36* (±0.25) 5.51	1.2** (±0.08) 0.0[a]	6.17** (±0.03) 414.17
3	480.45** (±3.14) 0.0[a]	411.19*** (±2.65) -14.42	239.78** (±2.17) 0.0[a]	174.95*** (±0.75) -27.04	4.57*** (±0.24) 0.0[a]	5.73** (±0.29) 25.38	1.8** (±0.05) 0.0[a]	7.19*** (±0.04) 299.44
4	622.94*** (±2.85) 0.0[a]	570.94*** (±2.46) -8.39	313.92*** (±1.04) 0.0[a]	220.75* (±0.71) -29.68	4.45*** (±0.11) 0.0[a]	5.91*** (±0.31) 32.81	1.9* (±0.04) 0.0[a]	7.73** (±0.05) 306.84

a The values indicate percentage variation relative to initial values. Values are mean of three experiments and with ±SD. Significantly different from the control at *P<0.05, **P<0.01 ***P< 0.001 by one-way ANOVA with Tukey-Kramer multiple comparisons test.

Similarly it is evident from the Table 5 that the turbidity of samples increased with increase in concentration of zinc in samples. The turbidity was observed to be increased on 10th day as compared to its values on 0[th] day. The minimum turbidity observed was 0.18 NTU for control sample on 0 day while the maximum turbidity observed was 7.37 NTU at 4% sample on 10[th] day. The turbidity increased considerably on 10[th] day as compared to the turbidity on 0[th] day and it continued following the increasing trend with increase in zinc concentration in samples (Figure 2). The increase in turbidity of the samples after 10 days of treatment with *Azolla* might be due to the secretions and fall of dead biomass in solution of pots. On the other hand Gudekar and Trivedi (1989) reported 59.54% reduction of turbidity in treatment of engineering industry waste with water hyacinth.

Hardness and acidity

It is evident from the Table 6 that hardness of samples decreased at all concentration after 10 days of treatment when compared to initial concentration. Among the treatment trays maximum decrease (-34.28%) was observed with 1% of electroplating effluent. Similarly acidity of the samples when compared with initial and final concentration of the same % solutions, it was also decreased showing good treatment ability of *Azolla*. Maximum reduction was observed in 1% effluent with a reduction of 26.60%. Though it was in serial increase with increasing percentage of the effluent, its reduction was remarkable as compared to the initial values and is of importance with the aim of the preset study.

Table 6. Reduction in hardness, acidity, sodium and potassium of zinc plating effluent grown with *A. caroliniana* (after 10 days).

Zinc	Hardness		Acidity		Na		K	
Concentration (%)	Initial	Final	Initial	Final	Initial	Final	Initial	Final
	47.95	30.15	55.16	40.49	11.71	11.19	7.21	4.56
Control	(2.89)	(1.23)	(1.56)	(2.56)	(0.56)	(0.82)	(0.48)	(0.76)
	0.0[a]	-37.12	0.0[a]	-26.60	0.0[a]	-4.44	0.0[a]	-36.75
	69.46**	45.65**	64.24*	50.65**	13.25	11.98*	9.75*	7.93
1%	(3.65)	(2.56)	(2.89)	(1.64)	(1.02)	(1.05)	(0.98)	(0.48)
	0.0[a]	-34.28	0.0[a]	-21.16	0.0[a]	-9.58	0.0[a]	-18.67
	84.64**	70.36*	73.48***	60.84*	14.73**	12.43**	11.54***	8.74***
2%	(4.56)	(3.15)	(1.57)	(3.45)	(0.99)	(0.84)	(0.49)	(0.47)
	0.0[a]	-16.87	0.0[a]	-17.20	0.0[a]	-15.61	0.0[a]	-24.26
	107.78*	90.26***	105.19***	80.41***	16.27***	13.75*	17.88**	11.26**
3%	(3.48)	(2.48)	(3.42)	(3.56)	(1.15)	(0.21)	(0.69)	(0.43)
	0.0[a]	-16.26	0.0[a]	-23.56	0.0[a]	-15.49	0.0[a]	-37.02
	134.49***	113.91**	117.75**	100.56***	23.16*	18.76***	36.42*	17.34***
4%	(4.64)	(3.48)	(4.15)	(3.46)	(1.43)	(0.53)	(1.49)	(1.14)
	0.0[a]	-15.30	0.0[a]	-14.60	0.0[a]	-19.0	0.0[a]	-25.39

a The values indicates percentage variation relative to initial values. Values are mean of three experiments and expressed in mg/l with ±SD. Significantly different from the control at *P<0.05, **P<0.01 ***P< 0.001 by one-way ANOVA with Tukey-Kramer multiple comparisons test.

Sodium (Na+) and potassium (K+) content

Sodium content of the samples also decreased after 10 days of treatment with *Azolla.* It was obvious that Na^+ content was in increasing trend from 1 to 4% on zero day and 10[th] day as there was external addition of the effluent (Table 6). On the other hand it is also clear that the experimental species has ability to treat Na^+ and K^+ from electroplating effluent. Maximum reduction for Na^+ content was observed at 4% of the sample and was 19% while for the K^+, the same concentration was treated efficiently by *Azolla* biomass with 25.39% reduction as compared to zero day concentration. Overall decrease in initial concentration of Na^+ and K^+ indicate that *Azolla* has better ability of survival under toxic effluent. In short, floating macrophyte *Azolla* is one of the efficient candidates for sodium and potassium removal. A reduction of 56% of sodium and 99.39 per cent potassium respectively with 100 per cent and 25 per cent concentration of textile industry waste treated with *Eichhornia crassipes* after four days of treatment had been recorded by Trivedi and Gudekar (1987). Water hyacinth (*Eichhornia crassipes)*, is a floating macrophytes whose appetite for nutrients and explosive growth rate has been put to use in cleaning up municipal and agriculture wastewater (Gupta, 1980). In the present study, sodium and potassium content of effluent treated with *Azolla* is normal and as per earlier studies (Lumpkin and Plucknett, 1980). Moreover, it was determined that more growth of biomass was nearly led to more removal of metals (Khosravi, 2005).

Table 7. Reduction in BOD and COD of zinc plating effluent grown with *A. caroliniana* (after 10 days).

Electroplating effluent %	BOD Initial	BOD Final	COD Initial	COD Final
Control	1.6 (±0.15) 0.0^{a}	1.4 (±0.16) -12.50	4.5 (±0.29) 0.0^{a}	4.2 (±0.86) -6.67
1	3.4* (±0.18) 0.0^{a}	2.9* (±0.18) -14.71	7.8 (±0.37) 0.0^{a}	6.2** (±0.67) -20.51
2	4.8*** (±0.2) 0.0^{a}	3.9*** (±0.19) -18.75	11.6** (±0.54) 0.0^{a}	7.8*** (±1.01) -32.76
3	5.2** (±0.18) 0.0^{a}	4.6*** (±0.28) -11.54	18.9*** (±1.24) 0.0^{a}	15.6 (±1.04) -17.46
4	6.7*** (±0.24) 0.0^{a}	5.9** (±0.24) -11.94	22.6*** (±1.28) 0.0^{a}	16.8*** (±1.32) -25.66

a The values indicates percentage variation relative to initial values. Values are mean of three experiments and expressed in mg/l with ±SD. Significantly different from the control at *P<0.05, **P<0.01 ***P< 0.001 by one-way ANOVA with Tukey-Kramer multiple comparisons test.

Biological oxygen demand and chemical oxygen demand

Marked reduction in BOD and COD of all final treated samples was observed when compared with zero day values (Table 7). Value for BOD was decreased maximally by 18.75% in the effluent concentration of 2% while the decrease in COD was by 32.76% in the same effluent concentration after 10 days treatment period. Greater reduction in values of COD was observed as compared to BOD values and might be due to the more amounts of dissolved minerals present in the samples than organic matter. Significance decrease in concentration of BOD was observed by various scientists when the effluents from various industries were treated with constructed wetlands. Different effluent samples from *Eichhornia* sp., *Salvinia* sp., *Pistia* sp. *and Typha* sp. showed marked reduction in BOD where maximum reduction of BOD in undiluted effluent was with *Eichhornia* sp. based CWs (490.9%) followed by *Typha* sp. based CWs (400%) (Santos et al., 1987). An evaluation of ten systems utilizing surface flow wetlands by Conley (1991) showed BOD removal rates ranged from 64 to 96%. Kirzhner et al. (2008) working on phytoremediation reported that BOD of industrial effluents was removed by 65 to 70% after four days of retention period by floating macrophytes.

Zimmels et al. (2006) noticed 360% reduction in COD in a pilot study with *Eichhornia crassipes* and *Pistia stratiotes* for treatment of urban sewage in Israel. Zhang et al. (2007) reported that the efficiency of COD removal varied a lot for various species to different contaminants. In case of undiluted effluent, *Typha* sp. based CWs removed maximum amount of COD. Treatment of textile dye using anaerobic baffled reactor by wetland plants removed 70-90% COD (Bell and Buckley, 2003). The reduction in COD and BOD can result in an increase in dissolved oxygen concentration of wastewater (Brix, 1998) and is an important outcome in relation with the species used to treat the effluent.

Zinc accumulation and bio-concentration factor (BCF) in the *A. caroliniana*

The toxic effect of metals on physiological functioning within plants is connected to their accumulation in different plant tissues (Liu et al., 2005). Several cases of accumulation of heavy metals such as Zn, Cu, Pb, Cd, Ni and Cr, have been thoroughly studied in several wetland plant species, such as *E. crassipes*, *Typha latifolia, Spartina alterniflora* and *Phragmites australis* (Liu et al., 2005). An increase in zinc (Zn^{++}) accumulation in *A. caroliniana* with increasing concentrations of zinc in the hydroponics was observed (Table 8). The maximum accumulation of Zn^{++} by *A. caroliniana* was 5610 mg/kg at 2% of the effluent concentration while it was minimum at 4% of effluent concentration by a value of 1420 mg/kg. In the present study increase in concentration of zinc in *A. caroliniana* at elevated levels might be due to their response to favor metal tolerance and involvement in maintenance of plant growth for overall development. Kumar et al. (1989) have also investigated elemental composition of certain aquatic plants by EDAX and found high level of heavy metals such as Al, Si, Mn and Fe accumulated in *Vallisnaria spiralis*, *Hydrilla verticillata and Azolla pinnata*. Accumulation of 110, 841 and 1260 mg Zn^{++}/kg (dry mass) was recorded by Khosravi et al. (2005) in *Azolla filiculoides.*

Table 8. Accumulation of zinc in *A. caroliniana* at various treatments and their effect on bio-concentration factor.

Sr. No.	Zn^{++} content	Zinc concentrations (%)				
		Control	1%	2%	3%	4%
1	Initial concentration (mg/l)	3.7(±0.23) 0.0[a]	7.68*(±0.24) 107.57	11.58**(±0.86) 212.97	17.42***(±0.48) 370.81	20.18*(±1.01) 445.41
2	Residual Water 'x' (mg/l)	2.61(±0.03) 0.0[a]	3.06(±0.05) 17.24	4.11***(±0.05) 57.47	1.18***(±0.02) -54.47	0.52***(±0.01) -80.08
3	*A. caroliniana* 'y' (mg/kg)	1816 (±7.09) 0.0[a]	3616*** (±12.66) 99.12	5610***(±5.0) 208.92	3108**(±7.37) 71.15	1420**(±0.58) -21.81
4	Bio-concentration factor (x/y)	696.85(±7.83) 0.0[a]	1183.35***(±23.45) 69.81	1365.13**(±18.84) 95.90	2641.97***(±38.51) 279.13	2732.08***(±52.54) 292.06

a The values indicates percentage variation relative to initial values. Values are mean of three experiments and expressed in mg/l with ±SD. Significantly different from the control at P<0.05, **P<0.01 ***P< 0.001 by one-way ANOVA with Tukey-Kramer multiple comparisons test.

Bioconcentration factor (BCF) is a useful parameter to evaluate potential of the plants in accumulating metals and this value is calculated on a dry weight basis. The change in BCF of *A. caroliniana* was also studied in present investigation to know capacity of *A. caroliniana* to concentrate zinc from varied effluent concentrations at the end of treatment. As a fact larger BCF implies better phytoaccumulation capability and tissues with BCF greater than 1,000 are considered high, and less than 250 low, with those between classified as moderate (Zayed et al., 1998). The BCF values were increased serially as per increasing concentration of zinc effluent. All effluent concentrations showed higher accumulation capacities as the BCF values were more than 1000. 4% of the effluent recorded with higher BCF as 2732.08 while minimum for control treated biomass was observed by a value of 696.85. In the present study increasing BCF shows that the *A. caroliniana* has capacity to accumulate Zn^{++} through liquid medium after ten days exposure period which is also in accordance with other studies (Table 9). The sequestration of heavy metals in plants is achieved mainly by absorption and accumulation mechanisms (Dhir, 2010). Arora (2006) studied chromium BCF range between 243 and 4617 for the three species of azolla in which *A. microphylla* showed highest bioconcentration potential. They further concluded that the azolla species can be exploited for treatment of tannery and chromium contaminated wastewaters. Moreover, it was determined that the more growth of biomass was nearly led to more removal of metals (Khosravi, 2005). While Jain et al. (1990) reported the BCF value for Zinc in *Azolla pinnata* as only 44.

Table 9. Bioconcentration factors (BCF) for zinc in various plants used for phytoremediation.

Sr. No.	Plant Species	Bioconcentration Factor	Reference
1	*Azolla caroliniana*	2732.08	This study
2	*Eichhornia crassipes*	788.9	Xiaomei et al. (2004)
3	*Azolla pinnata (root)*	12,000	Sela et al. (1989)
4	*Myriophyllum exalbescense*	1,640	Franzin and McFarlane (1980)
5	*Ricciocarpus natans*	3,700-8,800	Caines et al.(1985)
6	*Elodea nuttalli*	3,000	Nakada et al. (1979)
7	*Lemna polyrrhiza*	44	Jain et al.(1990)

Conclusions

Azolla caroliniana can be used to treat zinc from the polluted waters which accumulate in its biomass. *A. caroliniana* showed maximum efficiency of accumulation of Zn^{++} up to 4% of concentration. Photosynthetic pigments of *Azolla* were also observed to increase under the influence of zinc and other contents of effluent. The reduced level of electrical conductivity and pH after treatment period also shows the ability of experimental species to treat the effluent. The TDS, COD and BOD were also reduced after treatment period. The decrease in sodium and potassium content along with other selected water parameters mainly hardness, acidity were definitely associated with the use of effluent contents for the normal growth and development under the influence of toxic effluent. *Azolla* with its BCF values can be considered as a high accumulator for Zn. Effluents containing zinc at slightly higher concentration can be treated with the use and growth of *Azolla* biomass.From the present study, the floating hygrophyte *A. caroliniana* based treatment has proved as a promising tool for the treatment of zinc electroplating effluent at lower dilutions. Its favorable growth and growing biomass has favored rhizosphere activity in the liquid medium thereby enhancing dissolved substances and metal uptake. It may also be possible to use the biomass as a biofertilizer after harvesting. We also suggest the need to understand processes that affect metal availability, metal uptake and translocation in azolla. Detailed investigations and understanding of various aquatic species, accumulation of different metals under influence of various effluents should be studied with respect to physiological changes associated with the plants and their ability to treat the industrial and domestic wastewaters.

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References

Abraham, E., G. Rigo, G. Szekely, R. Nagy, C. Koncz and L. Szabados. 2003. Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in arabidopsis. Plant Mol. Biol. 51:363-372.

Alicia, P. D. N., J. N. Jaun, O. Oscar and C. Richard. 1994. Quantitative importance of particulate matter retention by the roots of *Eichhornia crassipes* in the Parana flood plain. Aquatic Bot. 47:213-223.

Amelia, K. K. 2001. The potential for constructed wetlands for waste water treatment and reuse in developing countries: a review. Ecological Eng. 16:545-560.

Andra, S. S., D. Sarkar, K. C. Makris, C. P. Mullens, S. V. Sahi and S. B. H. Bach. 2010. Synthesis of phytochelatins in vetiver grass upon lead exposure in the presence of phosphorus. Plant Soil, 171-185.

Antunes, P. M., G. M. Watkins and J. R. Duncan. 2001. Batch studies on the removal of gold (III) from aqueous solution by *Azolla filiculoides*. Biotechnol. lett. 23:249-251.

Armstrong, G. A. and J. E. Hearst. 1996. Carotenoids 2: Genetics and molecular biology of carotenoid pigment biosynthesis. FASEB J. 10:228-237.

Arnon, D. I. 1949. Copper enzyme in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.

Arora, A., S. Saxena and D. K. Sharma. 2006. Tolerance and phytoaccumulation of chromium by three Azolla species. World J. Mircobiol. Biotechnol. 22:97-100.

Ashton, P. J. and R. D. Walmsley. 1976. The aquatic ferm *Azolla* and *Anabaena* symbiot. Endeavour 35:39-45.

Bates, L. S., R. P. Waldren and T. D. Teare. 1973. Rapid determination of proline for water stress studies. Plant Soil. 39:205-207.

Bell, J. and C. A. Buckley. 2003. Treatment of textile dye using anaerobic baffled reactor. Water. 29:432-437.

Brix, H. 1998. Denmark. In Constructed wetlands for wastewater treatment in Europe. In: J. Vymazal, H. Brix, P. F. Cooper, M. B. Green and R. Haberl (Eds). pp.123-152. Backhuys Pub. Leiden.

Caines, L. A., A. W. Watt and D. E. Wells. 1985. The uptake and release of some trace metals by aquatic bryophytes in acidified waters in Scotland. Environ. Pollut. Ser. B 10: 1-18.

Chen, G. and Z. Djuric. 2001. Carotenoids are oxidized but do not protect lipids from peroxidation in unilamellar liposomes. FEBS Lett. 505:151-154.

Choo, T. P., C. K. Lee, K. S. Low and O. Hishamuddin. 2006. Accumulation of chromium (VI) from aqueous solutions using water lilies (*Nymphaea spontanea*). Chemosphere 62:961-967.

Conley, L. C., R. I. Dick and L. W. Lion. 1991. An assessment of the root zone method of wastewater treatment. Res. J. Water Pollution Control Fed. 63:239-247.

DeHerralde, F., C. Biel, R. Save, M. A. Morales, A. Torrecillas, J. J. Alarcon and M. J. Anchez-Blanco. 1998. Effect of water and salt stresses on the growth, gas exchange and water relations in *Argyranthemum coronopifolium*. Plants. Plant Sci.139:9-17.

Dhir, B. 2010. Use of aquatic plants in removing heavy metals from wastewater. Int. J. Environ. Eng. 2:185-201.

EI-Ghamery, A. A., M. A. EI- Kholy and M. A. Abou EI-Yousser. 2003. Evaluation of cytological effects of Zn^{++} in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L. Mutation Res. Genetic Toxicol. Environ. Mutagenesis 537:29-41.

Fisher, J. P. 1988. Wastewater treatment using aquatic plants. In: Rao Bhamidimarri (Ed.), pp.34-44. Alternative waste treatment systems, Elsevier Applied Science, Palmerston North, New Zealand.

Folin, O. and W. Denis. 1915. A calorimetric estimation of phenols and phenol derivatives in urine. J. Biol. Chem. 22:305-308.

Franzin, W. G. and G. A. McFarlane. 1980. An analysis of the aquatic macrophytes, *Myriophylhum exalbescens*, as an indicator of metal contamination of aquatic ecosystems near a base metal smelter. Bull. Environ. Contam. Toxicol. 24:597-605.

Fricke, W., G. Akhiyarova, W. Wei, E. Alexandersson, A. Miller, P. O. Jellbom, A. Richardson, W. Tobias, L. Schreiber, D. Veselov, G. Kudoyarova and V. Volkov. 2006. The short-term growth response to salt of the developing barley leaf. J. Exp. Bot. 57:1079-1095.

Galun, M. 1987. Removal of metal ions from aqueous solutions by *Pencillium* biomass: Kinetic and uptake parameters. Water Air Soil Pollution. 33:359-371.

Gauba, N., T. O. Mahmooduzzafar, S. Siddiqui and M. U. Iqbal. 2007. Leaf biochemistry of *Lycopersicon esculentum* Mill. At different stages of plant development as affected by mercury treatment. J. Env. Biol. 28:303-306.

Ghaly, A. E., M. Kamal and N. S. Mahmoud. 2004. Phytoremediation of aquaculture wastewater for water recycling and production of fish feed. Env. Technol. 14:1011-1016.

Groudev, S. N., M. V. Nicolova, I.I. Spasova, K. Komnitsas and I. Paspaliaris. 2001. Treatment of acid mine drainage from a uranium deposit by means of a natural wetland. Paper presented at the ISEB Phytoremediation Conference, Leipzig, Germany pp.146-148.

Gudekar, V. R. and R. K. Trivedi. 1989. Effect of surface area covered by water hyacinth. Ind. J. Env. Prot. 19:103-107.

Gupta, G. C. 1980. Use of water hyacinth in wastewater treatment. Int. J. Env. Health 43:80-82.

Hagemeyer, J. 1999. Ecophysiology of plant growth under heavy metal stress. In: M. N. V. Prasad and J. Hayegemeyer (Eds.). pp.157-181. Heavy Metal Stress in Plants.

Jain, S. K., P. Vasudevan and N. Jha. 1990. *Azolla pinnata* R. Br. and *Lemna minor* L. for removal of lead and zinc from polluted water. Wat. Res. 24:177-183.

Jarunee, J., U. Kenji and M. Hiroshi. 2003. Differences in physiological responses to NaCl between salt-tolerant *Sesbania rostrata* Brem. and Oberm. and non-tolerant *Phaseolus vulgaris* L. Weed Biol. Manage. 3:21-27.

Kamal, A., A. E. Ghaly, N. Mahmoud and R. Cote. 2004. Phytoaccumulation of heavy metals by aquatic plants. Env. Int. 29:1029-1039.

Kenneth, E., K. E. Pallett, and A. J. Young. 2000. Carotenoids. In: G. Ruth Alscher and L. John (Eds.). pp.60-81. Antioxidants in Higher Plants, Hes CRC Press.

Khosravi, M. M., G. Taghi and R. Rakhshaee. 2005. Toxic effect of Pb, Cd, Ni and Zn on *Azolla filiculoides* in the international Anzali wetland 2:35-40.

Kirk, J. O. T. and R. L. Allen. 1965. Dependence of salinity stress on the activity of glutamine synthatase and glutamate dehydrogenase in triticale seedlings. Polish J. Env. Studies 14:523-530.

Kirzhner, F., Y. Zimmels and A. Gafni. 2008. Effect of evapotranspiration on the salinity of wastewater, treated by aquatic plants. Rev. Environ. Health 23:149-166.

Kitoh, S., N. Shiomi and E. Uheda. 1993. The growth and nitrogen fixation of *Azolla filliculoides* Lam. in polluted water. Aquat. Bot. 46:129-139.

Kojo, S. 2004. Current Medicinal Chemistry. Curr. Med. Chem. 11:1041-1064.

Kumar N. J. I., S. S. Sreenivas and B. C. Rana. 1989. EDAX- analysis of mud of four ponds from central Gujarat. Ind. Bot. Contractor. 6:75-76.

Kumar, P. and R. Chandra. 2004. Detoxification of distillery effluent through *Bacillus thuringiensis* (MTCC 4714) enhanced phytoremediation potential of *Spriodela polyrrhiza* L. Schliden. Bull. Env. Cont. Toxicol. 73:903-910.

Lasat, M. M. 2002. Phytoextraction of toxic metals: a review of biological mechanisms. J. Environ. Qual. 31:109-129.

Liao, S. W. and W. L. Chang. 2004. Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. J. Aquat. Plant Management. 42:60-68.

Lu, X., M. Kruatrachue, P. Pokethitiyook and K. Homyok. 2004. Env. Sci. Tech. Manage. Res. 30:93-103.

Lumpkin, T. A. and D. L. Plucknett. 1980. *Azolla*: botany, physiology and use as green manure. Econ. Bot. 34:111-153.

Lutts, S., V. Majerus and J. M. Kinet. 1999. NaCl effects on proline metabolism in rice (*Oriva sativa*) seedlings. Physiol. Plant. 105:450-458.

Mahmood, Q., P. Zheng, E. Islam, Y. Hayat, M. J. Hassan and R. C. Jilaniand. 2005. Lab scale studies on water hyacinth (*Eichhornia crassipes* Marts 24. Solms) for biotreatment of textile wastewater. Caspian J. Env. Sci. 3:83-88.

Mane, A. V., G. D. Saratale, B. A. Karadge and J. S. Samant. 2011. Studies on the effects of salinity on growth, polyphenol content and photosynthetic response in *Vetiveria zizanioides* L. Nash. Emirates J. Food Agric. 23:59-70.

Mishra, K. K., U. N. Rai and O. Prakash. 2004. Bioconcentration and phytotoxicity of Cd in *Eichhornia crassipes*. Env. Monit. Assess.130:237-243.

Mishra, K. K., U. N. Rai and O. Prakash. 2007. Bioconcentration and phytotoxicity of Cd in *Eichhornia crassipes*. Env. Mon. Asses. 130:237-243.

Munns, R. 2003. Comparative physiology of salt and water stress. Plant Cell Env. 25:239-250.

Nakada, M., K. Fukaya, S. Takeshita and Y. Wada. 1979. The accumulation of heavy metals in the submerged plant (*Elodea nuttallii*). Bull. Env. Cont. Toxicol. 22:21-27.

Oncel, I., Y. Kele and A. S. Ustun. 2000. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Env. Poll. 107:315-320.

Parida A., A. Das and P. Das. 2002. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguira perviflora*, in hydroponic cultures. J. Plant. Biol. 45:38-36.

Peters, G. A. and J. C. Meeks. 1989. The Azolla-Anabaena symbiosis: basic biology. Ann. Rev. Plant Physiol. Plant Mol. Biol. 40:193-210.

Santos, E. J., E. H. B. C. Silva, J. M. Fiuza and T. R. O. Batista. 1987. A high organic load stabilization pond using water hyacinth-A Bahia experience. Water Sci. Tech. 19:25-28.

Saratale, R. G., G. D. Saratale, J. S. Chang and S. P. Govindwar. 2011. Outlook of bacterial decolorization and degradation of azo dyes: a review. J. Taiwan Inst. Chem. Eng. 42:138-157.

Schat, H., R. Vooijs and E. Kuiper. 1996. Identical major gene loci for heavy metal tolerances that have independently evolved in different local populations and subspecies of *Silene vulgaris*. Evolution 50:1888-1895.

Sela, M., J. Gary and E. Tel-Or. 1989. Accumulation and the effect of heavy metals on the water fern *Azolla filiculoides.* New Phytol. 112:7-12.

Singh, O. V., S. Labana, G. Pandey, R. Budhiraja and R. K. Jain. 2003. Phytoremediation: An overview of metallic ion decontamination from soil. Appl. Microbiol. Biotechnol. 61:405-412.

Spearot, R. M. and J. V. Peck. 1984. Recovery process for complexed copper-bearing rinse. Waters Environ. Prog. 3:124-129.

Stratford, H. K., T. H. William and A. Leon. 1984. Effects of heavy metals on water hyacinths (*Eichhornia crassipes*). Aquat. Toxicol. 5:117-128.

Toth, S. J., A. L. Prince, A. Wallace and D. S. Mikkelsen. 1948. Rapid quantitative determination of eight mineral elements in plant tissues by systematic procedure involving use of a flame photometer. Soil Sci. 66:456-466.

Trivedi R. K. and V. R. Gudekar. 1987. Treatment of textile industry waste using water hyacinth. Water Sci. Tech. 19:103-107.

Valavanidis, A., T. Vlahogianni, M. Dassenakis and M. Scoullos. 2005. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Env. Saf. 64:178-189.

Wagner, G. M. 1997. *Azolla*: A review of its biology and utilization. Bot. Rev. 63:1-26.

Watanabe, I. 1984. Use of symbiotic and free living blue-green algae in rice culture. Outlook Agric. 13:166-172.

Xiaomei, Lu, M. Kruatrachue, P. Pokethitiyook and K. Homyok. 2004. Removal of cadmium and zinc by water hyacinth, *E. crassipes*. Sci. Asia. 30:93-103.

Zayed, A., S. Gowthaman and N. Terry. 1998. Phytoaccumulation of trace elements by wetland plants. I. Duckweed. Env. Qual. 27:715-721.

Zhang, X., L. I. U. Peng, Y. Yang and W. Chen. 2007. Phytoremediation of urban wastewater by model wetlands with ornamental hydrophytes. J. Env. Sci. 19:902-909.

Zhao, M., J. R. Duncan and R. P. Van Hille. 1999. Removal and recovery of zinc from solution and electroplating effluent using *Azolla Filiculoides*. Wat. Res. 33:1516-1522.

Zimmels, Y., F. Kirzhner and A. Malkovskaja, 2006. Application of *Eichhornia crassipes* and *Pistia stratiotes* for treatment of urban sewage in Israel. J. Env. Manage. 81:420-428.