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Remediation of inorganic substances and heavy metals through aquatic macrophytes from flashlight manufacturing industry effluents

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ABSTRACT

The macrophytes *Eichhornia crassipes* and *Pistia stratiotes* were applied for the removal of heavy metals (Hg, Zn, Pb, Mn) and inorganic substances (nitrate, phosphate, total suspended solid, total dissolved solid, chlorides, sodium, potassium) from the effluents of a flashlight manufacturing industry. Both *E. crassipes* and *P. stratiotes* were grown in different concentrations of effluent v/v (water/water) i.e. 100%, 75%, 50%, 25% and 0% for 35 days in an experimental setup. Our observations and results reveal that *E. crassipes* and *P. stratiotes* are highly efficient in removing heavy metals (Hg, Zn, Pb, and Mn), inorganic substances (nitrate, phosphate) and solids from the effluents. The present study also reiterates the viewpoint that *E. crassipes* and *P. stratiotes* have a high potency in metal transport system with metals being concentrated in the roots and shoots of the macrophytes. Considering the translocation factor, the results suggest that heavy metals were largely retained in the roots of *P. stratiotes* and the shoots of *E. crassipes*.

Key-words: *Eichhornia crassipes*, Heavy metals, Macrophytes, *Pistia stratiotes*.

INTRODUCTION

Freshwater is precious for the existence of life (including humans) on our planet. However, as a consequence of rapid industrialization, large amounts of toxic pollutants have been and continue to be released into freshwater resources (e.g., lakes, rivers). Amongst these pollutants, heavy metals are considered to be of major concern due to their persistent and accumulative nature (Change et al. 2009, Yadav et al. 2009, Sood et al. 2012, Saxena et al. 2016). Thus, it is deemed necessary to develop environmentally and economically feasible technology to remove toxins from water. Although, various conventional technologies are being used for the removal of heavy metals from wastewater, such as chemical precipitation, reverse osmosis, ion exchange and electrochemical deposition, but none of them are considered to be cost effective and eco-friendly as compared to Phytoremediation (i.e., removal of metals using aquatic plants). Many previous studies have considered aquatic plants that include but not limited to *Lemna minor*, *Eichhornia crassipes*, *Hydrilla verticillata*, *Ipomoea aquatica*, *Azolla* (*A. caroliniana*, *A. filiculoides*, and *A. pinnata*), *Pistia stratiotes*, *Eleochari sacicularis*, *Salvinia* (*S. herzogii*, *S. minima*, *S. natans*, *S. rotundifolia*), *Phragmites australis*, *Typha* (*T. latifolia*, *T.* angustifolia), *Spirodela polyrhhiza* to be useful for the efficient removal of heavy metals from industrial effluent (Toet et al. 2005, Mishra et al. 2008, Dhir 2009, Jadia & Fulekar 2009, Mohammad et al. 2010, Rahman & Hasegawa 2011, Sood et al. 2012, Priya et al. 2013; Sasmaz et al. 2015, Rezania et al. 2016).

Thus, taking into consideration previous studies that suggest a higher efficiency in removing heavy metals and other inorganic pollutants from industrial effluents by macrophytes (Mohammad et al. 2010, Priya et al. 2013), we here made observations in order to record the efficiency of Macrophytes (particularly *E. crassipes* and *P. stratiotes*) in removing toxins (particularly heavy metals, nitrates, phosphates and solids) from the effluents of a battery and flashlight manufacturing industrial unit(s) located about ½ kilometre away from Aishbagh, Lucknow, Uttar Pradesh, India. *Eichhornia crassipes*is is an aquatic macrophyte popularly known as "*water hyacinth*" while *Pistia stratiotes*is is an aquatic macrophyte of the family Araceae, commonly known as "water lettuce". It should also be noted that previous studies have shown a large number of pollutants such as: Hg, Pb, Zn, and Mn, phosphate, nitrate, TDS, TSS, chloride and EC within the effluents generated from the industrial unit(s), Aishbagh, Lucknow (Kumar et al. 2009, Vishnoi et al. 2013, Kumar et al. 2016).

MATERIAL AND METHODS

Sampling, experimental setup and methods for data analysis

The wastewater sample for carrying out the present investigation was collected from outlet pipes situated about ½ kilometre away from the above-mentioned industry in pre-washed (with distilled water) 10 litres capacity plastic containers. A total of 25 effluent water samples were collected for undertaking the present investigation. The plant samples for *Eichhornia crassipes* and *Pistia stratiosis* were collected from the Gomti river, Lucknow. After proper field collections, the macrophytes *Eichhornia crassipes* and *Pistia stratiosis* were introduced to a hydroponic system (containing tap water) for 7 days for acclimatization prior to exposure to the experimental setup.

The experimental setup was set in the net house of the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh. All the 25 containers were initially setup in a staircase-like pattern (i.e., in five rows and five columns). Initially, individual rows were introduced with 100, 75, 50, 25 and 0% concentration of effluents. Subsequently, after 7 days, all the effluents were transferred with fresh plants in individual containers.

The pH, EC and temperature were analysed electronically; alkalinity, acidity, chloride, hardness were measured using the titrimetric method; TS, TDS and TSS were measured using the gravimetric method; Na, K and Ca were analysed by using the flame photometer; BOD was analysed by 5 days incubation; COD was analyzed by the reflex digestion method and the total nitrogen was measured using the Kjeldalh method. The determination of Zn, Pb, Mn, Hg was carried out by atomic absorption spectrophotometer. All the plant samples were washed, cut into small pieces and air dried prior to homogenisation by mortar and pestle. Subsequently, 1g of sample was digested using aquaregia (HNO_3 and $HClO_4$ acid in 3: 1 ratio for plants and 5:1 ratio for water v/v) until the samples became clear and white. Further, the samples were diluted with 0.1 N HNO_3 and filtered using a 0.45 µm filter paper.

The various factors that were examined in the present study include:

Removal Efficiency (RE): The concentration of effluents (in terms of presence of heavy metals) was examined at a 7 day interval and the removal efficiency were calculated following Tanhan et al. 2007 and Khellaf & Zerdaoui 2009.

$$RE = \frac{Ci - Cf}{Ci} \times 100$$

Where Ci is the initial concentration and Cf is the final concentration of heavy metals.

Translocation factor (TF): The translocation factor (TF) is the ratio of heavy metals in the roots and shoots of the plants, and determines the potential of the plant for heavy metal accumulation (Barman et al. 2000; Gupta et al. 2008).

$$TF = \frac{\text{Conc. of metal in shoots}}{\text{Conc. of metal in roots}}$$

Enrichment coefficient (EC): Enrichment coefficient is the ratio of the metal accumulated in plants and the metal concentration in water (see Sasmaz et al. 2008). The enrichment coefficient (EC) was calculated following Rahmani and Sternberg (1999).

$$\text{Enrichment coefficient} = \frac{\text{Metal accumulated in plants (rot or shoot)}}{\text{Metal concentration in water}}$$

Finally, the dataset was analysed using the One Way ANOVA test so as to compare the physiochemical properties of wastewater and metal accumulation in plant tissues with time. A Post-hoc tukey's test ($p<0.05$) was used to compare the metal concentration in both *Eichhornia crassipes* and *Pistia stratiosis*. It should be noted that all the calculations were carried out using the SPSS 16.0 software.

RESULTS AND DISCUSSION

Physio-chemical analysis of waste water effluent

Physio-chemical analysis of effluents collected from the flashlight industries is shown in Table 1. Temperature, pH and EC were important parameters for biochemical activities of water. The pH of the sample was 8.83, which means that the effluents are slightly alkaline in nature and slightly higher compared to the permissible levels of 6-8 (WHO 2004). High pH values in water bodies have been reported to affect the aquatic system and change the toxicity of other pollutants in one form or the other. Tafesse et al. (2015) reported that pH is the most important water parameter for deciding the quality of waste water effluents. It also enhances the heavy metal toxicity at a particular level. Electrical conductivity of waste water sample was 18.1 S/cm, which is 7 times higher than the permissible level (1000μS/cm, (WHO 2002), and indicative of a higher level of pollution. EC is the combination of dissolved and dissociated substance which depends on temperature, dissociation, concentration and migration of ions in the electric field, however, it does not provide inferences on the types of ions present in the sample (Chowdhury et al. 2013). The high conductivity value (i.e. high salt concentration) in the effluents can increase the salinity of the receiving river, which means higher EC that adversely affects the aquatic biota. Further, high concentrations of salt is quite likely hazardous to human health (Adeolu 2011). The analyzed samples also contain a higher concentration of total solid, total dissolved solid, and total suspended solid i.e., having the values of 24183.62, 22.74 and 243878.16 mgl^{-1}, respectively. Further, the TDS is composed mainly of carbonates, bicarbonates, chlorides, phosphates, nitrates, calcium, magnesium, potassium and organic substances. It has been shown in previous studies that an increased heavy metal concentration in waste water is indicative of an increase in the suspended solids concentrations (Lokhande et al. 2011, Chowdhury et al. 2013). Alkalinity, acidity, chloride and total hardness were recorded as 452.43, 142.24, 6686.43 and 300mgl^{-1}, respectively. It should be noted that water with a higher alkalinity is corrosive and is unsuitable for many purposes. Source of acidity in water is due to dissociation of acid and carbon dioxide in water. Higher acidity decreases pH, through this unwanted heavy metals separate in water. Chloride is necessary for aquatic life but higher concentration negatively affects reproduction rate, species mortality and changing characteristics of ecosystem (Rajkumar & Kim 2006). Calcium (Ca^{++}) and magnesium (Mg^{++}) are main cation and $CO_{2,}$ $^{-}HCO_3^-$, SO_4^- and NO_3^- anions are chiefly responsible for total hardness (Sahu et al. 2008; Kumar et al. 2012).

Sulphate, nitrate and phosphate were recorded as 0.61, 0.70 and 0.60 mg/l and sodium, potassium and calcium recorded as 157.66, 46.33 and 75.66 mgl^{-1} respectively. BOD and COD of the sample are very high (as compared to prescribed standard of WHO i.e. 30mgl^{-1} for BOD and 250 mgl^{-1} for COD) which is recorded as 253.73 and 4754.14mg/l. Higher BOD means low oxygen is available for microorganisms in the water bodies. COD is dissolved oxidizable organic matter including non-biodegradable matter present in waste water. High COD levels indicate toxic conditions and the presence of biologically resistant organic substances (Can et al. 2014). Observed COD value in effluents is much higher than permissible level of WHO 2002. The concentration of Pb, Zn, Mn, and Hg was 2.37, 1.75, 1.55, and 6.34 mgl^{-1} respectively. In the effluents, Hg concentration was high. Hg compound is used as electrolyte to prevent corrosion. It releases hydrogen which extends the life and improves the performance of the batteries. Mercuric oxide is also used as cathode in Hg oxide batteries.

Table 1. Physico-chemical analysis of flashlight manufacturing industry effluent (FMIE), Lucknow, India

Parameters	Effluent (FMIE)	Tap water	WHO Standard
pH	8.83± 0.15*	6.90±0.15*	6.5- 8.5
Temperature (°C)	23.56±0.57*	24.10±0.15**	-
EC (S/m)	18.1±0.49*	864±80.52* (µS/cm)	1000-2000 (µS/cm)
Alkalinity	452.43±12.49*	180.34±16.19***	100
Dissolved Oxygen	ND	0.67±0.15*	8
BOD	253.73±21.40*	4.07±0.32*	30
Total Hardness	306±32.75*	228.5±13.37****	300
Acidity	142.24±12.22*	_	-
Total solids	24183.62±1145.59****	694.11±54.41*****	-
Total Dissolved solid	24160.81±1266.05*****	693.4±56.23*****	500
Total Suspended solid	22.74± 0.54*	0.58±0.006*	-
Chlorides	6686.43± 378.27***	32.8±0.56**	200
Phosphate	0.60±0.001*	-	
Carbon dioxide	24.07±0.21*	-	-
Nitrate	0.70±0.05*	0.02±0.002*	-
COD	4754.14± 240.25**	-	250
Sulphate	0.61±0.07	2.1±0.05*	200
Sodium	157.67±1.52*	2±0.17*	-
Potassium	46.33±1.52*	2.33±0.37*	-
Calcium	75.67±5.8*	7±0.58*	75
Heavy metals (mg L^{-1})			
Zn	1.75±0.003*	0.015±0.001*	05
Hg	6.36± 0.05*	0.001±0.009*	0.001
Mn	1.56±0.002*	0.004±0.001*	0.1
Pb	2.37±0.03*	0.02±0.006*	0.05

Physico- chemical parameter (all parameter units are mg L^{-1}except pH, Temperature and EC), *ND= Not detected

Physico-chemical properties of waste water after treatment

After 35th day of treatment with *Pistia stratiotes* and *Eichhornia crassipes* some physico-chemical properties shows differences in TDS, TSS, chloride, phosphate and nitrate, but some properties i.e. pH, EC, sodium, potassium did not show much difference from initial concentration to final concentration. pH of the sample measured 8.83 to 8.37 in 100% , 8.73 to 8.3 in 75%, 8.7 to 8.2 in 50% , 8.6 to 8.2 in 25% and 7.9 to 8.7 in 0% with *Pistia,* while 8.8 to 8.4, 8.7 to 8.3, 8.7 to 8.3 8.58 to 8.2 and 7.75 to 8.1 with *Eichhornia* respectively after 35th days of treatment. pH of the sample changes slightly due to photosynthesis and other metabolic activities of plants like absorption of some soluble nutrients by roots and acidifying them, via emitting hydrogen ions (H^+) with exchange of cation and to produce carbon dioxide and organic acids (Lu et al. 2010, Vymazal 2007, Gikas & Tsihrintzis, 2012). After 35 days, EC of the sample was 3.53, 3.53, 2.85, 2.23 and 0.84 for *Pistia* while in *Eichhornia* it was found to be 3.53, 3.23, 3.42, 2.33 and 0.84 in 100, 75, 50, 25 and 0% of sample. Changes in the EC showed the removal of nitrogen and phosphorus salt dissolved in the sample (Souza et al. 2013). Removal efficiency of *Pistia stratiotes* for potassium is 12.16 %, sodium 16.57% and phosphate 29%, while *Eichhornia* removed 38.01% potassium, 16.15% sodium, 21.47% chloride, 35% phosphate and 50% of nitratein average after 35days. *Pistia* removed 83.18%, 80.64%, 88.79% and 98.19 % of TDS and 89.02%, 78.76%, 90.38% and 94.65% of TSS and

E. crassipes also removed 97.04%, 97.27%, 95.74% and 94.32% TDS and 92.90%, 90.84, 76.12% and 75.05% TSS from 100%, 75%, 50% and 25% sample concentration respectively. The average TDS values after treatment is still very high indicating that phytoremediation process is not sufficient to remove TDS below permissible level. Chloride also shows good removal efficiency by *Pistia,* it removed 55.35%, 55.81%, 51.72%, and 61.11% chlorides from 100%, 75%, 50% and 25% water respectively, which is slightly higher as compared to *Eichhornia* (54.92%, 55.81%, 48.27% and 21.47%).

Both macrophytes play an important role and create positive situation for various physico- chemical and biological processes, which contribute in the removal of PO_4^- and NO_3^-. After treatment NO_3^- and PO_4^- also showed less concentration removal as compared to others. *Pistia* after 35 days in 100%, 75%, 50%, 25% and 0% removed 0.42, 0.30, 0.22 and 0.18 mgl^{-1} PO_4^- and 0.41, 0.32, 0.25 and 0.18 mgl^{-1}NO_3^-, *Eichhornia* removed 0.45, 0.36, 0.24, and 0.18 mgl^{-1} PO_4^- and 0.44, 0.35, 0.24 and 0.19 mgl^{-1} NO_3^-. Reddy (1983) and Gumbricht (1993) explain that plant uptake mechanism, under anaerobic condition denitrification process occurs which contributes to NO_3 decrease. The potential uptake rate of *Pistia stratiotes* is limited by its growth rate and concentration in the plant tissues (Vymazal 2007). Bindu et al. (2008) and Luca et al. (2014) reported that the removal of nitrates might be due to uptake by the plant roots and decreased concentration of nitrates could be due to the increased plant uptake rather than microbial denitrification.

The mechanism of phosphate removal could be reported to occur by sorption, complexation, precipitation and assimilation into plant biomass (Mishra et al. 2013). The better removal of phosphate in the planted system in the present study could be attributed to plant uptake and microbial assimilation. The main role of aquatic macrophytes with respect to removal of phosphate is direct uptake and provision of suitable conditions for microorganisms that use phosphate as a nutrient (Mbuligwe 2004). Korner & Vermaat (1998) observed that 50% of the phosphate is removed by the macrophytes and other 50% is removed by associated organisms and microorganisms. High metal removal rates are also common when aquatic plants are used for the remediation of waste water containing high concentrations of metals (Kao et al. 2001). TSS and TDS of samples also decrease after each stage of the experiment, where *E. crassipes* showed great efficiency as compared to *P. stratiotes* in overall experiment (Table 2).

Heavy metal removal efficiency and accumulation in roots and shoots of macrophytes

Hg, Zn, Mn and Pb are the four common metals found in flashlight industry and selected macrophytes removed these metals from waste water. The initial concentration of Zn was 1.75, Pb 2.27, Hg 6.33 and Mn 1.09 mgl^{-1} in the effluents.

Zinc is an essential micronutrient used in activation of enzyme and protein synthesis in plants, actively taken by roots (Du Laing et al. 2009). In the study *E. crassipes* removed 74.86, 76.13, 80.8 and 86.75%, while *P. stratiotes* removed 63.43%, 80.8%, 72.8% and 73.49% Zn from 100%, 75%, 50% and 25% effluent respectively, which shows *E. crassipes* is more efficient than *P. stratiotes*. Concentration of Zn in the roots of selected plants *E. crassipes* accumulates 1.13, 0.86, 0.58, 0.30 mgl^{-1} and *P. stratiotes* accumulates 1.02, 0.75, 0.52, 0.34 mgl^{-1}at 100%, 75%, 50% and 25% concentration compared to shoots in which *E. crassipes* accumulates 0.94, 0.66, 0.48, 0.33 mgl^{-1}, *P. stratiotes* accumulate 1.09, 0.82, 0.52, 0.22mgl^{-1} respectively which show that Zn is easily uptaken by roots. Zinc mobility is high within the *Eichhornia* and *Pistia* tissues so, translocation of Zn is at below ground organs.

Hg accumulation in macrophytes is highest due to its concentration in the effluents is higher as compared to other metals because mercuric compounds are used in manufacturing in flashlights. This is a non-essential element for plant growth. *Eichhornia* (>60%) and *Pistia* (>80%) removed Hg which indicates that these plants are also a good bio-accumulator of Hg. The highest removal efficiency shown by *P. stratiotes* (92.6%) at an effluent concentration of 50%, where the lowest efficiency denoted by *E. crassipes* (65.46%) at a concentration of 100% effluent after 35 days. Lenka

Table 2. Removal efficiency (RE%) of *Pistia stratiotes* and *Eichhornia crassipes* for removing inorganic contaminants and heavy metals.

	After 7 days		After 14 days		After 21 days		After 28 days		After 35 days	
	RE of *P. stratiotes*	RE of *E. crassipes*	RE of *P. stratiotes*	RE of *E. crassipes*	RE of *P. stratiotes*	RE of *E. crassipes*	RE of *P. stratiotes*	RE of *E. crassipes*	RE of *P. stratiotes*	RE of *E. crassipes*
Treatment with macrophytes in 100% effluent concentration										
pH	0.79	0.68	3.4	3.4	1.93	1.81	4.19	4.08	5.32	5.21
EC (mS)	3.03	2.75	0	0	1.1	0.83	12.67	12.67	2.75	2.75
TSS	48.21	69.74	76.75	84.04	89.79	87.75	97.4	88.68	98.33	95.68
TDS	26.57	27.43	41.43	56.61	51.89	67.3	61.58	71.17	67.5	77.3
Chloride	87.38	84.2	90.89	88.69	90.54	88.69	89.84	89.74	91.24	91.94
Sodium	3.2	1.06	-10.66	-10.45	-18.34	0.85	-13.22	-13.22	25.06	-7.25
Potassium	3.6	21.58	-1.45	9.91	-12.24	7.42	-3.6	22.3	-5.76	-18
Nitrate	20	20	0	-20	0	0	40	20	46	40
Phosphate	12.07	0	17.24	0	51.72	29.31	55.17	32.76	26.9	51.72
Hg	27.16	24.8	40.35	38.93	46.15	45.53	56.36	56.2	81.16	65.46
Pb	21.13	19.72	59.15	59.15	69.48	69.48	79.34	79.81	87.32	92.02
Mn	10.26	12.82	39.1	45.51	51.28	51.28	60.26	57.69	69.87	69.87
Zn	12.57	4	17.14	18.29	24.57	29.71	49.71	53.14	63.43	74.86
Treatment with macrophytes in 75% effluent concentration										
pH	0	0	2.63	2.63	2.63	2.63	3.78	3.78	4.93	4.93
EC (mS)	2.24	2.24	-48.72	-48.72	-33.97	-33.97	-33.65	-33.65	-13.14	-13.14
TSS	76.39	57.99	68.22	76.84	81.51	87.88	91.74	82.5	97.13	95.69
TDS	13.77	25.49	29.11	43.37	40.2	63.1	53.24	76.36	67.42	82.32
Chloride	46.51	28.99	48.84	32.56	51.16	30.23	48.84	44.19	55.81	55.81
Sodium	16.98	6.5	19.5	10.69	7.55	11.74	16.98	21.6	19.5	21.6
Potassium	5.36	17.27	7.14	25.46	28.57	23.41	23.21	17.86	26.79	2.38
Nitrate	-33.33	-33.33	-33.33	-33.33	-66.67	-66.67	0	20	0	33.33
Phosphate	8.33	16.67	18.75	2.08	54.17	8.33	54.17	52.08	27.08	54.17
Hg	30.52	27.07	47.93	44.14	54.31	53.45	57.59	62.59	82.24	69.66
Pb	37.16	34.43	62.84	65.57	74.86	73.77	78.69	78.14	89.62	91.26
Mn	16.09	11.49	27.59	24.14	35.63	33.33	44.83	44.83	68.97	70.11
Zn	12.9	20.65	20.65	32.26	33.55	37.42	60	60	72.26	76.13
Treatment with macrophytes in 50% effluent concentration										
pH	1.15	0.92	2.3	2.3	3.45	2.3	3.45	3.45	5.75	4.6
EC (mS)	12.04	5.86	27.78	-43.21	-0.62	-29.01	8.95	-28.7	12.04	-8.95
TSS	63.35	65.68	77.33	72.2	86.8	80.75	96.58	72.2	90.37	94.72
TDS	25.58	12.43	42.9	33.58	49.34	69.86	64.07	77.44	72.23	89.27
Chloride	28.27	37.93	28.27	37.93	62.07	31.04	55.17	27.59	51.73	48.28
Sodium	8.57	32.46	4.93	29.87	1.56	2.59	6.23	6.76	19.48	35.85
Potassium	-38.97	-111.73	-36.4	-119.84	-38.97	-119.1	-89.63	-89.67	5.18	-39.01
Nitrate	0	0	-50	-50	0	0	0	50	0	50
Phosphate	46.88	12.5	12.5	-21.88	43.75	50	43.75	46.85	31.25	43.75
Hg	31.74	32.46	41.29	29.59	52.03	48.45	54.42	58.71	92.6	73.03
Pb	32.88	37.67	60.27	61.64	82.19	81.51	84.25	84.25	89.73	90.41
Mn	19.7	18.18	24.24	27.27	48.48	40.91	63.64	56.06	71.21	75.76
Zn	9.6	9.6	24	24	30.4	40.8	63.2	67.2	72.8	80.8
Treatment with macrophytes in 25% effluent concentration										
pH	0.47	-1.51	0.81	1.16	-0.7	1.16	1.98	2.33	3.95	3.49
EC (mS)	13.6	-12.13	0	-70.59	2.94	-53.68	-0.37	-53.31	18.01	-29.78
TSS	89.43	61.99	96.95	74.59	97.36	85.77	97.36	86.18	94.72	95.53
TDS	10.77	23.3	66.51	42.2	80.24	50.33	82.64	75.21	83.96	86.15
Chloride	30.36	38.89	55.56	27.78	55.56	22.22	61.11	5.56	61.11	33.33
Sodium	13.11	21.71	14.93	21.71	38.46	14.92	19.45	2.72	2.25	38.91
Potassium	2.39	6.01	19.02	14.45	6.72	13.95	1.67	23.36	6.01	45.77
Nitrate	-50	0	-50	-50	0	0	50	0	50	45

Table 2 continued...

Phosphate	59.26	33.33	33.33	-18.52	37.04	55.56	44.44	51.85	33.33	51.85
Hg	47.09	28.75	55.66	32.42	72.17	44.04	79.2	67.89	85.32	71.56
Pb	2.74	1.37	54.79	53.42	69.86	69.86	84.93	83.56	83.56	84.93
Mn	31.25	12.5	21.88	28.13	40.63	40.63	46.88	50	84.38	78.13
Zn	13.25	21.69	18.07	42.17	20.48	69.88	71.08	80.72	73.49	86.75
Treatment with macrophytes in 0% effluent concentration										
pH	3.26	3.26	5.67	5.1	7.08	6.52	7.93	7.93	6.09	6.09
EC (mS)	6.52	6.52	11.96	11.96	6.52	6.52	1.09	1.09	8.7	8.7
TSS	23.08	23.08	23.08	23.08	30.77	30.77	-946.15	-946.15	46.15	46.15
TDS	3.35	11.45	21.81	20.09	13.61	27.65	16.85	46	30.89	66.52
Chloride	-185.76	-207.2	-114.33	-185.67	-114.73	-137.99	-114.73	-114.33	-114.7	-114.33
Sodium	14.16	-28.76	14.16	-28.76	18.45	14.16	14.16	-28.76	14.16	-57.51
Potassium	-36.43	-27.29	13.64	-7.64	54.57	-22.78	27.29	27.29	-27.29	-36.43
Nitrate	0	50	50	50	50	60	50	65	50	70
Phosphate	72	40	40	0	72	72	72	72	96	72
Hg	18.18	27.27	18.18	36.36	54.55	52.73	45.45	81.82	54.55	82.73
Pb	0	0	0	0	50	0	50	0	50	50
Mn	0	0	0	0	33.33	10	0	0	0	0
Zn	50	45	50	30	50	45	50	45	50	55

et al. (1990) explains bioconcentration factor of Hg in roots, which is dependent on time duration and concentration and also provides evidence that *E. crassipes* is a good candidate for Hg removal through adsorption and absorption. Skinner et al. (2007) undertook an experimental study on Hg uptake by using four macrophytes including *Pistia stratiotes*, *Eichhornia crassipes*, *Scirpus tabernaemontani* and *Colocasia esculenta*. These plants were treated with different concentration of mercury for 30 days. The result indicated that *Pistia stratiotes* and *Eichhornia crassipes* are more efficient accumulator of Hg as compared to others.

Mn is another essential metal for plants and also involved in photosynthesis and enzyme activity (Bonanno & Giudice 2010). Concentration of this metal in *Eichhornia* and *Pistia* varies each five weeks of the experiment. *Eichhornia* accumulates 0.76, 0.48, 0.30, 0.17 mgl^{-1}in roots and 0.78, 0.49, 0.30, 0.19 mgl^{-1}accumulates in shoots. *Pistia* accumulates higher amount of Mn in roots and shoots shown in figure 1. According to Demirezenv & Aksoy (2006), Mn easily moves within plants, and accumulates mainly in the green parts of plant organs. The results studied here have also revealed high Mn concentration in the top leaf section of the plant. Nonetheless, the highest content of Mn was found in the plant roots.

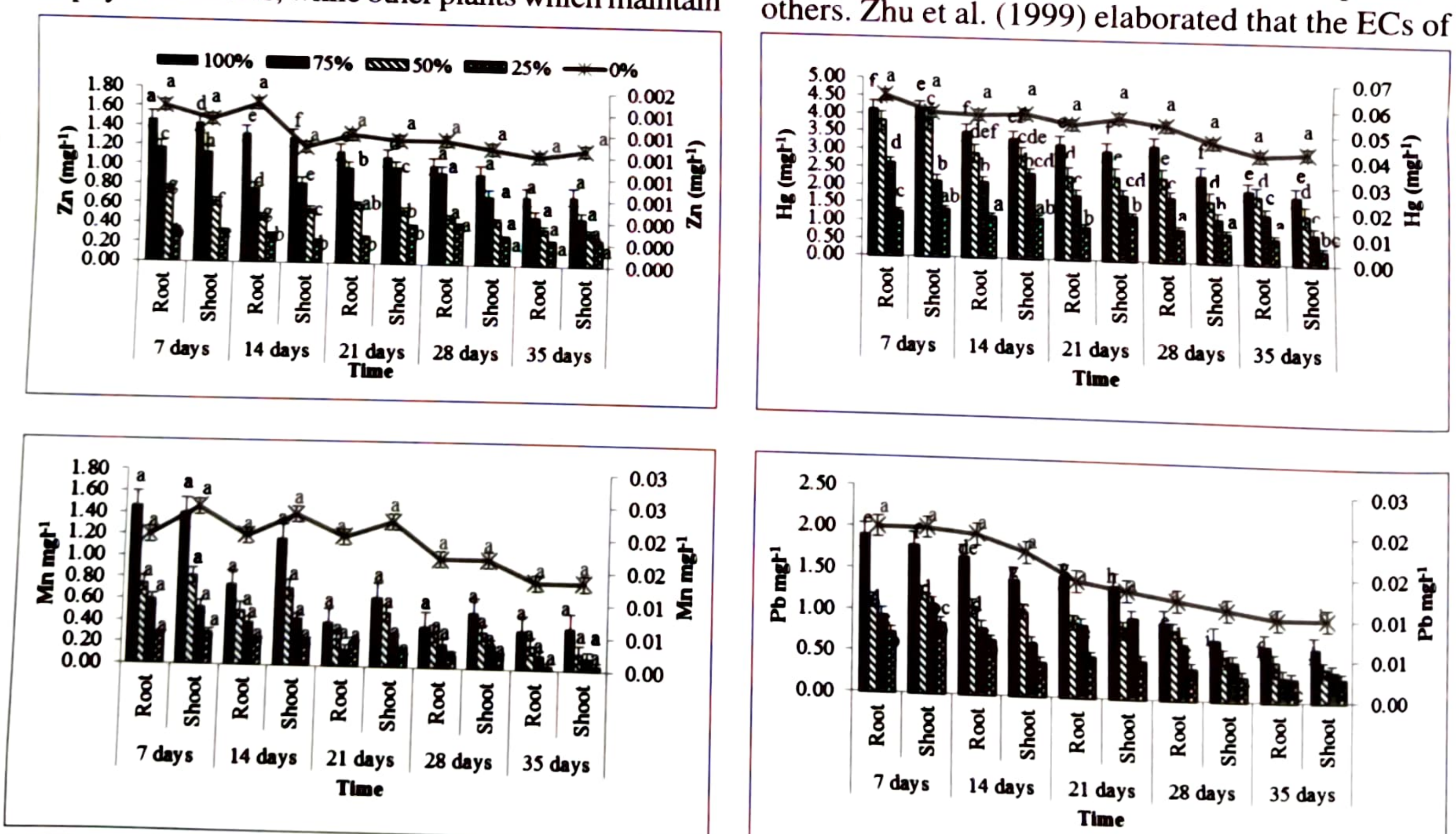
Pb is one of the toxic and non-essential metals for plant growth (Demirezen and Aksoy 2004). The concentrations of Pb, noted in *Eichhornia* is1.38, 1.05, 0.86, 0.71 mgl^{-1} in root and 1.07, 0.89, 0.78, 0.57 mgl^{-1} in shoot, in *Pistia,* 1.40, 1.05, 0.79, 0.51 mgl^{-1} in the roots and 1.19, 0.93, 0.75, 0.47 mgl^{-1} in the shoots respectively. *Pistia* have higher efficiency to accumulate Pb in their plant tissues shown in Fig. 1. Brix (1993) has observed that *E. crassipes* successfully used in waste water treatment system for improving the water quality by reducing the levels of nutrients (organic and inorganic). Thus, water hyacinth would probably have high tolerance and should be capable of removing large amounts of lead (Sutcliffe 1962). Similar findings were also reported by various researchers while working on *P. stratiotes* that was a hyper accumulator of Zn, Pb, Mn, Cu, Cr, Hg and Fe (Mishra & Tripathi 2008, Mokhtar et al. 2011, Aurangzeb et al. 2014, Galal & Farahat 2015, Chakraborty 2015). Odjegba and Fasidi (2004) undertook study on the accumulation of Ag, Cd, Cr, Cu, Hg, Ni, Pb, and Zn by using *P. stratiotes*. They observed tolerance, accumulation and toxicity response of plants against these metals. High concentration of these metals was accumulated in root tissue rather than in aerial parts. *P. stratiotes* indicated different tolerance level and accumulation capacities for different metals at similar treatment conditions.

The results revealed that the maximum efficiency is shown at 25 and 50% concentration of samples and minimum efficiency is shown at 100% and 75% concentration which indicates that higher concentration of metal in the samples slow down the phytoremediation process. All the data showed that the metal concentration in roots and shoots varied significantly on level ($p>0.05$) in both the plants.

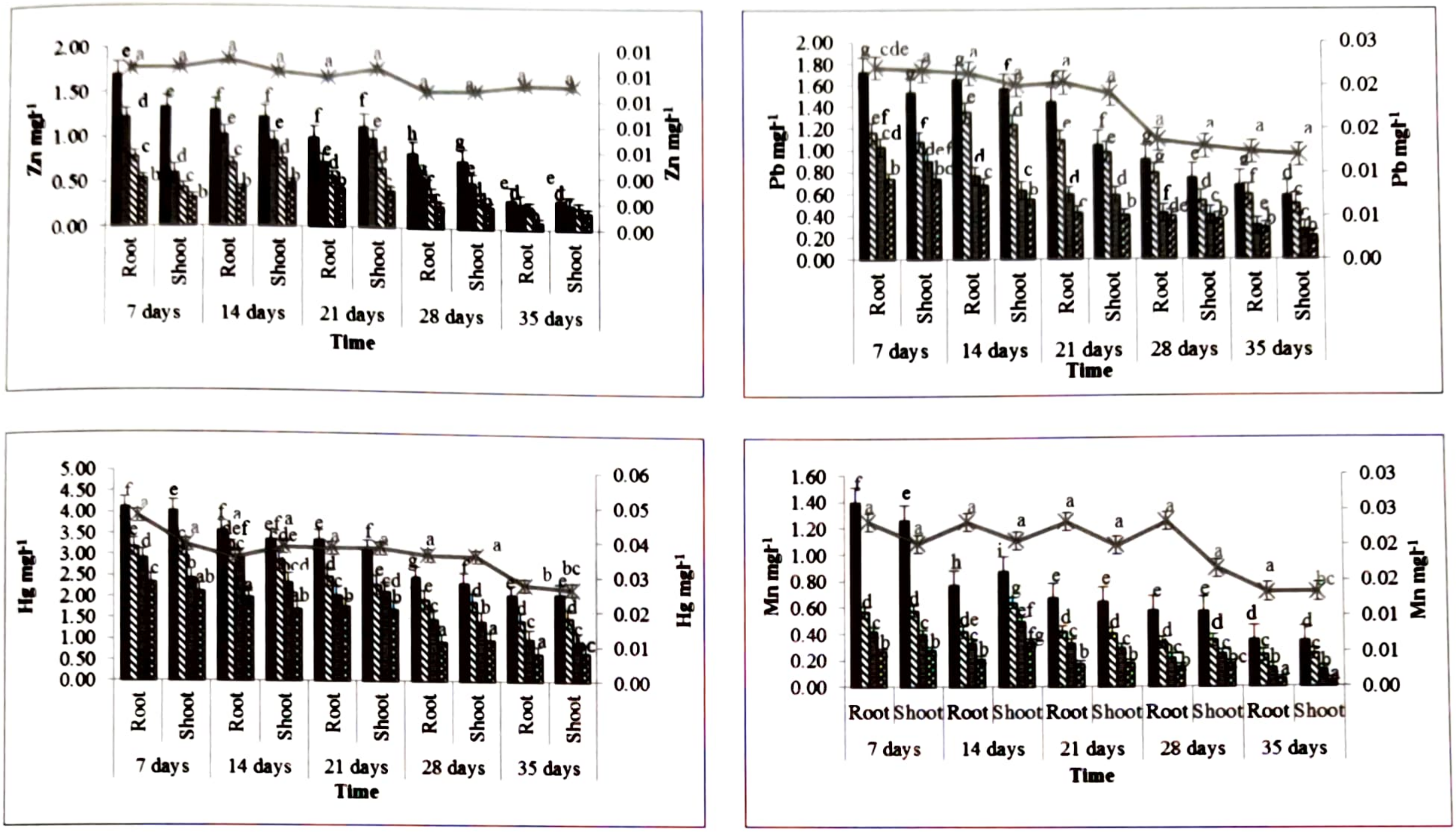
Translocation factor and enrichment coefficient

Recent results show that metal concentration among shoots and roots indicate translocation factor of macrophytes. Zhao (2002) explain that translocation factor with >1 indicates higher potency of plants in the metal transport system. All the metals Zn, Hg, Pb and Mn were accumulated in *E. crassipes* and *P. stratiotes* and it was found that all macrophytes showed higher translocation ability for Zn and Mn and lower for Pb and Hg, probably because Zn and Mn are essential elements for plants while Hg and Pb are not. Results showed that the roots of macrophytes have higher metal concentration. Kuperberg et al. (1999) explained that plants with higher concentration in shoots are known as phytoextractors, while other plants which maintain metal in roots are known as rhizofiltrator in phytoremediation classification. Thus, selected macrophytes translocation factor suits for rhizofiltration for Hg and Pb.

Enrichment coefficients (ECs) is the considering potential of macrophytes in accumulation of metals and basically depends on the soluble fraction of metals in water and their translocation in tissues and calculated on dry weight basis (Kumar et al. 2012, Krayem et.al. 2016). In general, when metals concentration in water decreases and accumulation in plants increases, the value of EC also increases. Jain et al (1990) found that ECs of *Lemna minor* and *Azolla pinnata* with Pb and Zn decreases with increasing metal concentration in solution. *E. crassipes* and *P. stratiotes* recorded higher enrichment coefficient value for Pb after 35 days which are: 5.00, 4.82, 4.90, 5.99, 1.36; 3.34, 3.10, 3.29, 3.44, 1.08 respectively while Hg showed lower value by both macrophytes. EC values of macrophytes is higher for Pb, which indicates that the accumulation capacity of plants is higher for lead as compared to others. Zhu et al. (1999) elaborated that the ECs of



Text-Figure 1. Accumulation of heavy metals i.e. Zn, Pb, Hg and Mn in root and shoot of *Pistia stratiotes* (One way ANOVA is performed to compare the means of different treatments at $p < 0.05$. Values followed by different letters are significantly differences between the treatments)



Text-Figure 2. Accumulation of heavy metals i.e. Zn, Pb, Hg and Mn in root and shoot of *Eichhornia crassipes* (One way ANOVA is performed to compare the means of different treatments at $p < 0.05$. Values followed by different letters are significantly differences between the treatments)

Eichhornia is higher for metals at low external concentration and they decrease when external concentration is increased. Enrichment coefficient and translocation factor values of *Eichhornia crassipes* and *Pistia stratiotes* of heavy metals are different in effluent concentration and time duration as shown in Table 3.

Table 3: Enrichment coefficient and translocation factor in *Eichhornia crassipes* and *Pistia stratiotes*

	Enrichment coefficient										Translocation factor				
	Root					Shoot									
Wastewater sample	7 days	14 days	21 days	28 days	35 days	7 days	14 days	21 days	28 days	35 days	7 days	14 days	21 days	28 days	35 days
	Eichhornia crassipes														
	Hg														
100%	0.90	1.02	0.96	0.89	0.93	0.86	0.86	0.90	0.80	0.94	0.96	0.85	0.93	0.90	0.84
75%	0.94	0.96	0.91	0.90	0.82	0.87	0.88	0.85	0.87	0.85	0.93	0.92	0.93	0.97	0.78
50%	1.03	0.98	0.94	0.84	0.89	0.86	0.79	0.98	0.82	0.82	0.84	0.80	1.04	0.98	0.64
25%	1.00	0.90	0.96	0.87	0.86	1.17	0.76	0.93	0.92	0.72	1.17	0.85	0.97	1.05	0.73
0%	0.02	0.01	0.04	0.02	0.01	0.02	0.02	0.02	0.18	0.01	1.05	1.53	0.60	9.94	0.73
	Zn														
100%	0.95	0.77	0.57	1.00	0.73	0.74	0.72	0.65	0.90	0.73	0.78	0.94	1.14	0.90	0.39
75%	0.99	0.81	0.53	0.86	0.74	0.48	0.76	0.74	0.80	0.71	0.48	0.94	1.39	0.93	0.49
50%	0.68	0.73	0.52	0.91	0.94	0.37	0.78	0.62	0.81	0.95	0.54	1.08	1.19	0.89	0.64
25%	0.63	0.86	0.65	1.00	0.81	0.38	1.00	0.62	1.00	1.89	0.59	1.16	0.95	1.00	0.78
0%	0.86	0.92	0.28	0.76	1.23	0.86	0.85	0.30	0.76	1.22	1.01	0.93	1.06	1.01	1.05

Table 3 continued...

	Pb														
100%	1.18	1.89	2.23	2.10	5.00	0.89	1.80	1.62	1.47	3.34	0.75	0.95	0.73	0.70	0.64
75%	0.96	2.13	2.29	2.28	4.82	0.98	1.95	2.06	1.37	3.10	1.32	1.17	0.96	0.72	0.66
50%	0.98	1.91	3.33	3.08	4.90	1.13	2.24	2.94	1.60	3.29	1.31	1.14	1.11	0.74	0.70
25%	1.02	2.91	2.07	5.95	5.99	1.02	3.40	1.80	1.75	3.44	1.41	1.29	1.70	0.54	0.68
0%	0.98	1.15	1.35	1.51	1.36	1.31	1.14	1.19	1.13	1.08	36.50	45.13	15.11	7.63	14.74
	Mn														
100%	1.03	0.90	0.88	0.86	0.74	0.93	1.26	0.84	0.85	0.73	0.90	1.40	0.96	0.98	0.60
75%	0.74	0.64	0.71	0.69	0.88	0.75	0.97	0.69	0.72	0.93	1.02	1.53	0.98	1.04	0.74
50%	0.77	0.68	0.84	0.74	0.83	0.74	1.02	0.75	0.82	0.76	0.95	1.51	0.89	1.12	0.57
25%	0.94	0.92	0.87	0.92	0.72	0.98	1.46	0.89	1.02	0.06	1.04	1.58	1.02	1.11	0.03
0%	7.78	7.78	0.88	0.78	0.40	6.78	6.89	0.75	0.56	4.00	0.87	0.89	0.86	0.71	5.71
	Pistia stratiotes														
	Hg														
100%	0.88	0.92	0.93	0.90	0.91	0.89	0.88	0.88	0.87	0.85	1.00	0.95	0.95	0.96	0.93
75%	0.94	0.96	0.87	0.86	0.92	0.95	0.94	0.88	0.69	0.68	1.01	0.98	1.01	0.80	0.73
50%	0.91	0.86	0.89	0.80	1.03	0.74	0.96	0.90	0.64	0.65	0.82	1.12	1.01	0.79	0.63
25%	0.49	0.58	0.47	0.48	0.65	0.56	0.57	0.65	0.50	0.37	1.14	0.98	1.39	1.03	0.57
0%	0.36	0.03	0.03	0.03	0.03	0.04	0.03	0.04	0.03	0.32	0.12	1.00	1.28	0.89	11.22
	Zn														
100%	0.96	0.89	0.87	1.15	1.11	0.93	0.87	0.83	1.06	1.12	0.98	0.97	0.95	0.92	1.01
75%	0.86	0.61	0.94	1.50	1.12	0.83	0.66	0.95	1.12	1.09	0.96	1.08	1.01	0.75	0.97
50%	0.65	0.49	0.69	1.65	1.06	0.54	0.68	0.62	1.02	0.99	0.83	1.40	0.90	0.62	0.94
25%	0.39	0.42	0.40	1.65	1.09	0.37	0.33	0.58	1.13	1.10	0.95	0.78	1.44	0.69	1.01
0%	0.10	0.10	0.08	0.09	0.10	0.09	0.09	0.11	0.08	0.11	0.92	0.86	1.30	0.94	1.12
	Pb														
100%	1.18	2.01	2.24	2.06	3.18	1.18	1.60	2.09	1.45	2.13	1.00	0.80	0.93	0.70	0.67
75%	0.95	1.97	2.35	2.27	4.06	1.04	1.33	2.55	2.14	2.62	1.09	0.68	1.08	0.95	0.64
50%	0.95	1.40	3.34	3.12	3.94	1.10	1.10	3.74	2.46	3.08	1.15	0.79	1.12	0.79	0.78
25%	1.02	2.02	2.27	3.54	2.28	1.21	1.27	2.11	2.72	2.32	1.18	0.63	0.93	0.77	1.02
0%	0.11	0.10	0.06	0.06	0.06	0.10	0.07	0.05	0.05	0.05	0.95	0.69	0.83	0.83	0.91
	Mn														
100%	1.00	1.23	0.84	0.83	0.83	1.04	0.78	0.52	3.37	0.81	1.04	0.63	0.62	4.05	0.97
75%	1.11	1.13	0.89	0.69	3.57	1.01	0.79	0.45	0.66	0.84	0.91	0.70	0.50	0.96	0.24
50%	1.00	0.86	0.75	0.86	0.61	1.12	0.78	0.43	0.88	0.69	1.12	0.91	0.57	1.02	1.12
25%	1.36	0.97	0.79	0.74	0.76	1.26	0.95	1.21	0.71	0.59	0.93	0.98	1.53	0.97	0.78
0%	7.78	7.11	8.75	5.56	4.00	6.67	7.00	7.50	5.56	3.40	0.86	0.86	0.94	0.99	0.99

CONCLUSION

The present study provides an approach for the removal of heavy metals (Hg, Zn, Mn and Pb) and other inorganic pollutants from flashlight industry effluents. The macrophytes proved highly effective in removing metals during a five week experiment. *Pistia* is capable to remove Zn and Pb and *Eichhornia* for removal of Mn from effluent water. These macrophytes improved the water quality by removal of nutrient nitrates and phosphates; it also removed TDS, TSS and chloride from the effluent water. Translocation factor also reveals that the roots of the macrophytes are better accumulators of metals and contain higher Zn, Pb and Hg while shoots of the macrophytes are better accumulators of Mn, further indicating a low transportation of metal from the roots to the shoots in the macrophytes investigated.

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