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Appraisal of the effect of industrial effluents on the chlorophyll, protein and heavy metals contents in water hyacinth (*Eichhornia crassipes* L.)

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ABSTRACT

The purpose of this study was to observe the effects of heavy metals on proteins, chlorophyll content and growth of *Eichhornia crassipes* L. in different concentrations of industrial effluents of district Shiekhupura. *Eichhornia crassipes* was grown for 15, 30 and 45 days in different concentrations of the industrial effluents (5, 10, 15, 20 and 100%) prepared with tap water. All physico-chemical parameters in the different concentrations of the industrial effluents were recorded. The values of these parameters increased with increasing the concentrations of the wastewater. Heavy metals analyzed in the different concentrations of effluents were in the order of Cr > Cd > Pb. The growth parameters like shoot length, root length, fresh weight of leaves and roots, dry weight of roots and leaves, were recorded in the different concentrations of industrial effluents. The maximum growth was observed in 10 and 15% concentrations and minimum in 100% (industrial effluents). The uptake of metals Cr, Cd, and Pb was recorded after 15, 30 and 45 days of the experiment. The protein and chlorophyll content were reported in *Eichhornia crassipes* after 45 days. Amount of chlorophyll decreased with the increase in concentration of effluents at all stages while protein contents increased with the increase in concentration of effluents and with time.

Keywords: Chlorophyll, Effluents, *Eichhornia*, Heavy metals, Protein

INTRODUCTION

Rapid large-scale development of industries/ industrialization along with the discharge of a variety of contaminated wastewater and untreated residential sewage effluents into the environment is one of the major sources of ecosystem pollution (Liu & Diamond, 2008; Gentry *et al*., 2018; Hamidi, 2010). The water is polluted due to weathering, ion exchange process, agricultural activities and anthropogenic activities (Arulbalaji & Gurugnanam, 2017). The presence of various pollutants in drinking water has the most serious threat to public health in Pakistan and is one of the major environmental problems (Azizullah *et al*., 2011). Both surface and groundwater in the country are contaminated with various toxic compounds including heavy metals from industries and from other resources (Azizullah *et al*., 2011). Pollution is a serious problem mostly in advanced countries, where the citizens, developing industry and improving their living standards or life style are creating environmental challenges (Uttara Aggarwal, 2012; Ameen & Mourshed, 2017).

The accumulation of heavy metals in the soil destroys the normal function of the soil ecosystem and plant growth (Khan *et al*., 2008; Zheng *et al*., 2008). Plants absorb various kinds of heavy metals when available in the soil or irrigation water (Fusconi *et al*., 2006). Lead is one of the maximum widely and lightly distributed trace metals which can affect soil, plant and animal health and it impairs various biological processes in flowers, elongation of root, transpiration, chlorophyll biosynthesis, and cellular development (Pourrut *et al*., 2011; Tangahu *et al*., 2011; Kumar *et al*., 2017). Remediation of heavy metals is essential for the protection and conservation of the environment (Glick, 2010). Phytoremediation is the usage of plants and their related microbes for environmental remediation. Farid *et al*. (2013) additionally reported the ameliorative consequences of phytoremediation on the contamination of wastewater. Many traditional methods are very costly and don't provide the satisfactory results. Phytoremediation, serves an ecological alternative, and has gained

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increasing attention since last decade as an emerging reasonable technique (Wani *et al.*, 2017).

Eichhornia crassipes L. (water hyacinth) is a member of family *Pontederiaceae* (pickerelweed). It is a fast growing perennial aquatic macrophyte, and is well known for its reproduction potential and ability to grow in severe contaminated waters. It is well studied as a water plant that could improve the effluent quality from polluted ponds and it could also be used for municipal, agricultural and industrial treatment (Dhote & Dixit, 2009). *Eichhornia crassipes* has ability to grow in sever conditions (Ndimele, 2012). Water hyacinth grows in a fresh water habitat for example marshes, shallow ponds, streams, rivers and lakes, which do not become saline during drought (Emeka *et al*., 2014). It has been selected for determining uptake efficiency and metal tolerance of potential phytoremediation species (Marchiol *et al*., 2004).

This study aims to remove heavy metals mainly chromium, cadmium and lead from waste-water by using an aquatic plant species, *Eichhornia crassipes*. The main purpose of the research was to (i) determine the pollution load in the sampling area by studying various physico-chemical parameters in the industrial effluents, and (ii) evaluate the proteins and chlorophyll contents in the plants of *Eichhornia crassipes* growing in different concentrations of the industrial effluents.

MATERIALS AND METHODS

Field surveys were conducted at the Saaim Thor pond, located at Lahore Sheikhupura Road in 2019. The plants were collected from different ponds, which were located besides Head Baloki Link Canal opposite crescent factory, Lahore Sheikhupura Road. The geographical position for sampling site was 31^{0}36'54.6"N 73^{0}53'33.5"E at an elevation of 645 feet as recorded by GPS (Model: Etrex H Garmin, Taiwan). The wastewater was collected from the drain (near the vicinity of Sheikhupura industries) in large plastic buckets, transported to the nursery of Department of Botany in University of Education, Township. The plants were transported to the wire-house to grow in nurseries then shifted to the greenhouse for experimental purposes.

The experiment was set up in wire-house in medium sized plastic pots in a Completely Randomized Design with factorial arrangement (Steel & Torrie, 1981) and was replicated six times; each replication was analyzed at fortnightly intervals. Effluents concentrations comprised 6 concentrations (0, 5, 10, 15, 20 and 100% respectively). Tap water was used as a control treatment. The experiment was carried out in the greenhouse of University of Education, Township, Lahore. Plants were harvested after 15, 30 and 45 days of the setup of the experiment.

The different pollution parameters were determine in the industrial effluents like pH, Electrical Conductivity (EC), and Total Dissolved Solids (TDS) by Multiparameter (Model: HI 9811-5). Carbonates, bicarbonates and chlorides were determined titrimetrically by following method of Saeed (1980). Chemical oxygen demand (COD) was measured by following a procedure of Greenberg *et al*. (1998) while Biological oxygen demand (BOD) of the different effluent concentrations was estimated using BOD Sensor Bottles. The Chlorophyll Content of plant material was determined by using Chlorophyll Meter (Model: SPAD-502 plus, Japan).

Different morphological parameters like root length, shoot length, number of leaves, number of roots, fresh weight (FW), and dry weight (DW) were observed at/after final harvest.

For metal assay, 25 ml of effluents was taken in a conical flask by adding 5 ml of nitric acid and 15 ml of perchloric acid. The content was heated on hot plate until the solution became clear. The solution was filtered and distilled water was added to make final volume up to 100 ml. Plants were harvested after 15, 30 and 45 days treatment and the roots and leaves were used to determine the concentration of heavy metals. After harvesting, both roots and shoots were dried separately on blotting paper at room temperature. Then the samples were dried in a microwave oven at 80 °C for 24 hours. The dried samples were grounded into fine powder using pestle and mortar to get a homogeneous mixture then shifted or stored to the small plastics bags for further analysis. The plants were acid digested according to the method describes by Greenberg *et al*. (1998). The estimation of heavy metals in all samples (effluent concentrations and plants) was accomplished by using Atomic Absorption Spectrophotometer (GBC, SAVAANT, AA Australia).

For protein estimation, Biuret Method of Racusen & Johnstone (1961) was performed with some modifications. In this regard 1 g fresh plant material (leaves and shoot) was crushed in liquid nitrogen with 0.5% (v/v) Triton X-100 and 0.1 g Polyvenyl polypirrolidone (Sigma Germany) in a fine powdered form to dissolve in 0.1 M phosphate buffer pH 7.8. The final volume of 3 ml of this extract was centrifuged (Sorval RB-5 refrigerated super speed centrifuge) at 14000 rpm at 4 °C for 30 minutes to get supernatant. The supernatant was used for the estimation of protein contents. The amount of protein was calculated from a standard curve, prepared from bovine serum albumin.

Statistical analysis

The data was statistically analyzed by using SPSS (Statistical Package for the Social Science) software version (20.0) and means were statistically compared at 0.05% probability level following F test.

RESULTS

The values of the physico-chemical properties increased with the increasing effluents concentrations; in the order of 0 < 5 < 10 < 15 < 20 < 100%. The pH, EC, and TDS were found to be maximum in 100% effluents concentration that indicates the pollution strength of the wastewater. The value of pH in 0% (Control) was 7.6 less than the 100% (industrial water, pH=8.2), because of more pollution in effluents than the tap water. EC was found to be highest (2,250 µS cm^{-1}) in 100% effluents concentration. Electrical Conductivity in 15% effluents concentration (930 µS cm^{-1}) was higher than in the 5% concentration (690 µS cm^{-1}). High values of EC show the presence of inorganic ions in the effluents. TDS were found to be lowest in 0% (290 mg L^{-1}). Industrial water contains more TDS because of contamination; therefore, 100% effluents concentration had much higher amount of TDS (1,090 mg L^{-1}) than the control (290 mg L^{-1}). It indicates the high-polluted area. Chemical oxygen demand was found to be maximum in industrial effluents 8,761 mg L^{-1} and lowest in tap water (150 mg L^{-1}). Biochemical oxygen demand in industrial effluents was 1,055 mg L^{-1} and 80 mg L^{-1} in tap water. Statistical analyses showed that the results of all above parameters were significant at $P \leq 0.05$. Carbonates were absent in all the industrial effluents and the amount of bicarbonates in the effluents varied. Bicarbonates in control were found to be 200 mg L^{-1} and in 5% were 1,245 mg L^{-1}. The high concentrations of bicarbonates were analyzed in the 15% (1,285 mg L^{-1}). The chloride amount increased from 22 mg L^{-1} (control) to 5,276 mg L^{-1} (100% effluents).

Industrial water contains toxic heavy metals Cr, Cd, and Pb and excessive amounts of these metals are toxic to plants. The amount of Cr was highest in industrial water 30,981 mg L^{-1} and was only (50 mg L^{-1}) in tap water. The amount of Cd in industrial effluents was maximum (15,432 mg L^{-1}) and was lowest in tap water (26 mg L^{-1}). Industrial water contained high amount of Cu (10,542 mg L^{-1}). The amount of Cu in 10% effluents concentration was 3,456 mg L^{-1} while in the 20% concentration, it was 7,542 mg L^{-1} (Table I).

Morphological growth parameters were observed in all concentrations of effluents (0, 5, 10, 15, 20 and 100%) after 15, 30 and 45 days old plants as shown in Table II. Statistical analysis showed that results of growth parameters were significant at $P \leq 0.05$. The morphological parameters decreased with the increasing concentrations of the effluents after 45 days of the experiment as shown in Fig. 1. Amount of chlorophyll decreased with the increase in concentration of effluents at all stages (Fig. 2). Highest amount of chlorophyll (5.556) was recorded in the control (0% effluents) and lowest at 100% (4.009) after 15 days. The amount of chlorophyll decreased gradually after some days. After 30 days, amount of chlorophyll was 3.333 µmol g^{-1} (FW) in 100% concentration as compared to 4,455 µmol g^{-1} FW at 0% concentration. Very low amount of chlorophyll was observed in 100% concentration (2.100 µmol g^{-1} FW) after 45 days because the plants were continuously in stressed condition due to high uptake of metals. Toxic metals may inhibit the synthesis of chlorophyll content in the plant tissues.

Maximum amount of heavy metals was recorded in maximum concentration of industrial effluents (Tables III-V). The metals content were in the order of, Cr > Cd > Pb. After 15 days, Cr concentration in the roots ranged from 15 mg kg-^{1} at 0% concentration to 8,098 mg kg-^{1} at 100% concentration and from 5 mg kg-^{1} to 5,012 mg kg-^{1}in the leaves at 0% and 100% concentrations, respectively (Table 3). The 0% concentration had the lowest amount of Cd in the roots (8 mg kg^{-1}), and the leaves (4 mg kg^{-1}). The amount of Pb ranged from 6 mg kg^{-1} in 0% concentration, to 3,245 mg kg^{-1} in the 100% concentration. In 5% concentration the amounts of Cr, Cd and Pb were 877, 322 and 210 mg kg^{-1}, respectively. This shows that the plants took higher amount of Cr than Cd and Pb.

Amounts of heavy metals recorded in the 30-days-old plants of water hyacinth are shown in Table IV. Highest amount of all metals was observed in 100% concentration in both roots and leaves; roots had higher metal contents than the leaves. The amount of Pb in 10% concentration in the roots was 678 mg kg^{-1} and 964 mg kg^{-1} in 20% concentration. The corresponding values in the leaves at the two concentrations were 312 mg kg^{-1} and 450 mg kg^{-1}, respectively. After 45 days, plants had the highest concentration of heavy metals (Cr, Cd~~,~~ and Pb), were in stressed conditions and started to wilt. Table V shows that *Eichhornia* *crassipes* in effluents (100%) accumulated high amount of Cr in the roots (6,321 mg kg^{-1}), and the leaves (3,031 mg kg^{-1}). The amount of Cr in 5% concentration in the roots was 2,087 mg kg^{-1} and 15,123 mg kg^{-1} in 100% concentration. The leaves of *Eichhornia* in 0% concentration had 16 mg kg^{-1} of Cr, which rose to 1,500 mg kg^{-1} and 2,256 mg kg^{-1} at 15 and 20% concentration, respectively. Higher metal concentration in the root tissues of the *Eichhornia crassipes* indicates the immobilization of metal by cell wall and extracellular carbohydrates, which appears to be an important defense technique, adopted by the plant. Statistical analyses showed that results of the uptake of heavy metals by *Eichhornia crassipes* were significant at P ≤ 0.05. The amount of Cr, Cd, and Pb increased consistently with the increase the concentration of industrial effluents. Thus, it is clear that *Eichhornia crassipes* absorbs heavy metal ions and can be used for reducing the pollutants taking place because of poisonous metallic ions within the effluents from different industries.

Amount of protein recorded in 45-old-day plants of water hyacinth is shown graphically in Fig. 3. Protein content increased with the increase in concentration of effluents and with time. Higher amount of protein was recorded in 100% effluents concentration after 45 days (2.0 mg g^{-1}) as compared to other concentrations.

Table I: Physico-chemical properties of different concentrations of industrial effluents.

Parameters	Industrial effluent concentrations						
	0%	5%	10%	15%	20%	100%	Effect of concentration on various parameters
pH	7.6 ±0.1	7.8 ±0.05	7.8 ±0.06	7.9 ±0.8	7.9 ±0.09	8.2 ±0.15	NS
EC (μs cm^{-1})	590 ±2.58	690 ±3.51	840 ±3.85	930 ±4.29	970 ±4.51	2250 ±5.16	S
TDS (mg L^{-1})	290 ±0.21	340 ±2.04	400 ±3.03	450 ±3.29	480 ±4.31	1,090 ±4.51	NS
COD (mg L^{-1})	150 ±2.08	1,200 ±3.03	2,091 ±3.37	3,321 ±4.60	4,872 ±5.06	8,761 ±8.96	S
BOD (mg L^{-1})	80 ±0.57	156 ±2.00	233 ±2.20	452 ±3.50	587 ±4.18	1,055 ±5.29	S
Carbonates (mg L^{-1})	0	0	0	0	0	0	
Bicarbonates (mg L^{-1})	200 ±0.62	1,245 ±1.12	1,276 ±1.3	1,285 ±1.34	308 ±2.13	515 ±3.10	S
Chlorides (mg L^{-1})	22 ±0.73	1,835 ±1.12	2,033 ±1.34	2,061 ±2.34	3,541 ±3.43	5,276 ±3.74	S
Cr (mg L^{-1})	50 ±1.73	8,122 ±2.12	10,987 ±2.32	13,021 ±3.53	18,076 ±4.00	30,981 ±4.36	S
Cd (mg L^{-1})	26 ±2.52	4,011 ±3.12	5,762 ±3.24	8,652 ±3.43	11,876 ±3.00	15,432 ±3.21	S
Cu (mg L^{-1})	18 ±2.08	2,342 ±3.10	3,456 ±3.23	7,432 ±3.90	7,542 ±4.34	10,542 ±4.76	S

0%= tap water
5%= 95ml of water with 5ml of effluents
10%=90 ml of water with 10ml of effluents
15%=85 ml of water with 15 ml effluents
20%=80 ml of water with 20ml of effluents
100%= industrial effluents
Values are mean ± Standard deviation from 6 replicates
Values are significant (S) at P ≤ 0.05 according to F test
S = Significant
NS Non-Significant

Table II: Morphological growth parameters of 45 days old *Eichhornia crassipes* L. plants grown in different concentrations of industrial effluents.

Growth parameters	Conc.	Days			Effect of concentration on various parameters
		After 15 days	After 30 days	After 45 days	
Root length (cm)	0%	30 ±0.10	33 ±0.25	34.5 ±0.29	NS
	5%	26 ±0.20	30 ±0.55	36 ±0.76	NS
	10%	27 ±0.35	32 ±0.64	31 ±1.53	S
	15%	28 ±0.52	31 ±0.71	32.5 ±1.75	S
	20%	29 ±1.53	33 ±1.59	31.5 ±2.36	S
	100%	24 ±2.52	25 ±2.00	30.5 ±2.75	S
Shoot length (cm)	0%	16 ±0.57	17 ±0.80	19 ±1.00	NS
	5%	13.5 ±0.71	12.5 ±0.87	10 ±1.52	S
	10%	14 ±0.81	10 ±1.05	11 ±2.25	S
	15%	15.5 ±1.15	14.5 ±1.75	13 ±2.64	NS
	20%	19 ±1.73	15 ±2.08	12 ±2.88	S
	100%	4.46 ±2.19	3.5 ±2.75	2.24 ±3.05	S
Fresh weight of leaves (g)	0%	25 ±0.56	10.51 ±0.77	24.51 ±1.32	S
	5%	25.3 ±0.75	13.03 ±1.00	11.63 ±2.59	S
	10%	23 ±0.76	27.14 ±1.60	20.97 ±3.24	NS
	15%	22 ±1.53	27.06 ±2.03	22.95 ±3.62	S
	20%	24 ±1.80	24.92 ±2.26	12.55 ±3.75	NS
	100%	20 ±2.64	8.83 ±2.84	6.7 ±3.81	S
Fresh weight of roots (g)	0%	24 ±0.34	22.18 ±0.61	17.39 ±0.50	S
	5%	19 ±0.50	12.04 ±0.64	13.74 ±1.43	S
	10%	20 ±1.00	16.7 ±0.89	16.56 ±1.58	NS
	15%	23 ±1.25	15.54 ±1.55	14.98 ±1.80	NS
	20%	17 ±1.53	7.29 ±1.69	9.06 ±2.05	S
	100%	13 ±2.08	5.86 ±2.00	3.27 ±2.26	S
No. of roots	0%	94 ±0.72	80 ±0.50	76 ±2.08	S
	5%	85	78	78	NS

		±0.81	±1.04	±2.36	
	10%	84 ±0.91	75 ±1.15	80 ±2.51	S
	15%	83 ±1.03	74 ±1.25	83 ±2.78	NS
	20%	82 ±1.06	73 ±1.28	72 ±3.05	NS
	100%	80 ±2.0	72 ±2.64	70 ±3.21	NS
No. of leaves	0%	10 ±0.58	11 ±0.61	7 ±0.76	S
	5%	8 ±0.76	9 ±1.15	5 ±0.90	NS
	10%	7 ±1.00	8 ±1.20	6 ±1.00	NS
	15%	6 ±1.53	7 ±1.31	8 ±1.25	NS
	20%	5 ±1.66	5 ±1.36	5 ±1.50	NS
	100%	4 ±2.0	3 ±2.08	4 ±1.53	NS
Dry weight (roots)	0%	2.1 ±0.61	1.45 ±0.36	5.96 ±2.07	S
	5%	1.9 ±0.74	1.36 ±0.43	2.34 ±2.14	NS
	10%	2.58 ±0.89	3.26 ±0.51	2.62 ±2.29	S
	15%	2.24 ±1.02	3.78 ±0.62	4.94 ±2.37	NS
	20%	1.61 ±1.67	2.78 ±0.85	1.4 ±2.53	S
	100%	1.02 ±1.96	1.96 ±1.36	1.84 ±2.93	S
Dry weight (leaves)	0%	2.5 ±0.09	1.43 ±0.03	3.85 ±0.94	S
	5%	2.36 ±0.36	2.13 ±0.16	1.93 ±1.17	S
	10%	2.37 ±0.60	2.94 ±0.77	2.38 ±1.37	S
	15%	2.72 ±0.75	2.63 ±1.13	2.33 ±2.22	S
	20%	2.5 ±1.04	2.96 ±1.32	1.9 ±2.91	S
	100%	1.4 ±1.60	1.09 ±2.22	0.95 ±3.00	NS

Values are mean ± Standard deviation from 6 replicates
Values are significant (S) at P ≤ 0.05 according to F test
S = Significant; NS Non-Significant

Table III: Amount of heavy metals in root and leaves of 15 days old plants of *Eichhornia crassipes* L. grown in different concentration of industrial effluents.

Days	Conc.	Plant parts	Metals (mg kg^{-1})			Effect of concentration on various parameters
			Cr	Cd	Pb	
After 15 days	0%	Root	15 ±0.50	8 ±0.57	6 ±1.00	S
		Leaves	5 ±0.60	4 ±0.76	2 ±1.26	NS
	5%	Root	877 ±0.68	322 ±0.95	210 ±2.00	S
		Leaves	580 ±0.72	266 ±1.00	143 ±2.52	S
	10%	Root	1,100 ±1.10	567 ±1.26	356 ±2.75	S
		Leaves	733 ±1.15	322 ±1.32	200 ±3.00	S
	15%	Root	1,398 ±1.53	754 ±1.62	477 ±3.21	S
		Leaves	1,011 ±1.04	358 ±1.75	289 ±3.44	S
	20%	Root	1,544 ±2.08	1,034 ±2.08	548 ±3.50	S
		Leaves	1,234 ±2.00	877 ±2.24	388 ±3.51	S
	100%	Root	8,098 ±2.64	4,097 ±2.30	3,245 ±3.60	S
		Leaves	5,012 ±3.00	2,367 ±2.51	2,265 ±4.52	S

Values are mean ± Standard deviation from 6 replicates
Values are significant (S) at $P \leq 0.05$ according to F test
S = Significant; NS Non-Significant

Table IV: Amount of heavy metals in root and leaves of 30 days old plants of *Eichhornia crassipes* L. grown in different concentration of industrial effluents.

Days	Conc.	Plant parts	Metals (mg kg^{-1})			Effect of concentration on various parameters
			Cr	Cd	Pb	
After 30 days	0%	Root	20 ±1.53	12 ±2.08	8 ±1.15	NS
		Leaves	8 ±2.64	8 ±1.00	4 ±1.53	NS
	5%	Root	1,022 ±2.75	567 ±1.52	402 ±2.08	S
		Leaves	834 ±2.76	312 ±1.75	256 ±2.36	S
	10%	Root	1,429 ±2.78	760 ±1.89	678 ±2.64	S
		Leaves	987 ±2.85	399 ±2.08	312 ±3.05	S
	15%	Root	1,800 ±2.89	900 ±2.52	877 ±3.21	S
		Leaves	1,234 ±3.05	508 ±2.64	300 ±3.50	S
	20%	Root	2,187 ±3.21	1,387 ±3.00	964 ±3.51	S
		Leaves	1,766 ±3.51	656 ±3.51	450 ±3.78	S
	100%	Root	12,785 ±3.60	5,234 ±4.04	4,863 ±4.07	S
		Leaves	6,872 ±4.16	3,108 ±4.36	2,893 ±4.58	S

Values are mean ± Standard deviation from 6 replicates
Values are significant (S) at $P \leq 0.05$ according to F test
S = Significant; NS Non-Significant

Table V: Amount of heavy metals in root and leaves of 45 days old plants of *Eichhornia crassipes* L. grown in different concentration of industrial effluents.

Days	Conc.	Plant parts	Metals (mg kg^{-1})			Effect of concentration on various parameters
			Cr	Cd	Pb	
After 45 days	0%	Root	30 ±0.58	20 ±1.00	15 ±1.73	S
		Leaves	16 ±0.76	12 ±1.25	8 ±1.15	NS
	5%	Root	2,087 ±1.00	923 ±1.63	733 ±1.52	S
		Leaves	1,032 ±1.26	528 ±1.69	355 ±1.80	S
	10%	Root	2,788 ±1.53	1,065 ±1.96	912 ±1.91	S
		Leaves	1,122 ±1.80	645 ±2.30	433 ±2.08	S
	15%	Root	2,845 ±2.14	1,234 ±2.51	1,098 ±2.64	S
		Leaves	1,500 ±2.15	818 ±3.00	566 ±2.92	S
	20%	Root	3,032 ±2.19	1,567 ±3.21	1,275 ±3.05	S
		Leaves	2,256 ±2.52	984 ±3.51	777 ±3.21	S
	100%	Root	15,123 ±2.64	8,981 ±4.04	6,321 ±3.78	S
		Leaves	7,863 ±3.21	5,239 ±4.35	3,031 ±4.00	S

Values are mean ± Standard deviation from 6 replicates
Values are significant (S) at $P \leq 0.05$ according to F test
S = Significant; NS Non-Significant

Fig. 1: *Eichhornia crassipes* L. grown in different concentration of effluents at the start of the experiment

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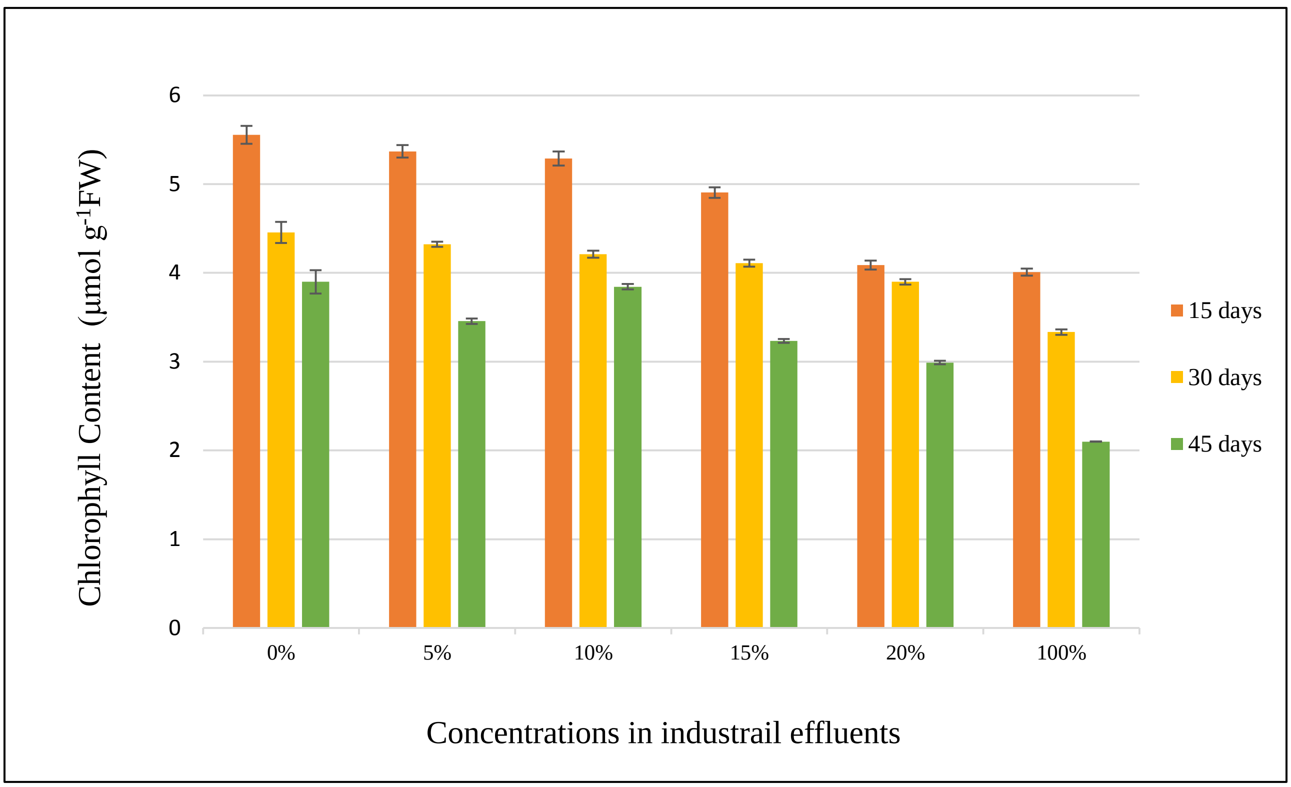

Fig. 2: Amount of chlorophyll content in leaves of 45 days old plants of *Eichhornia crassipes* L. grown in different concentration of industrial effluents.

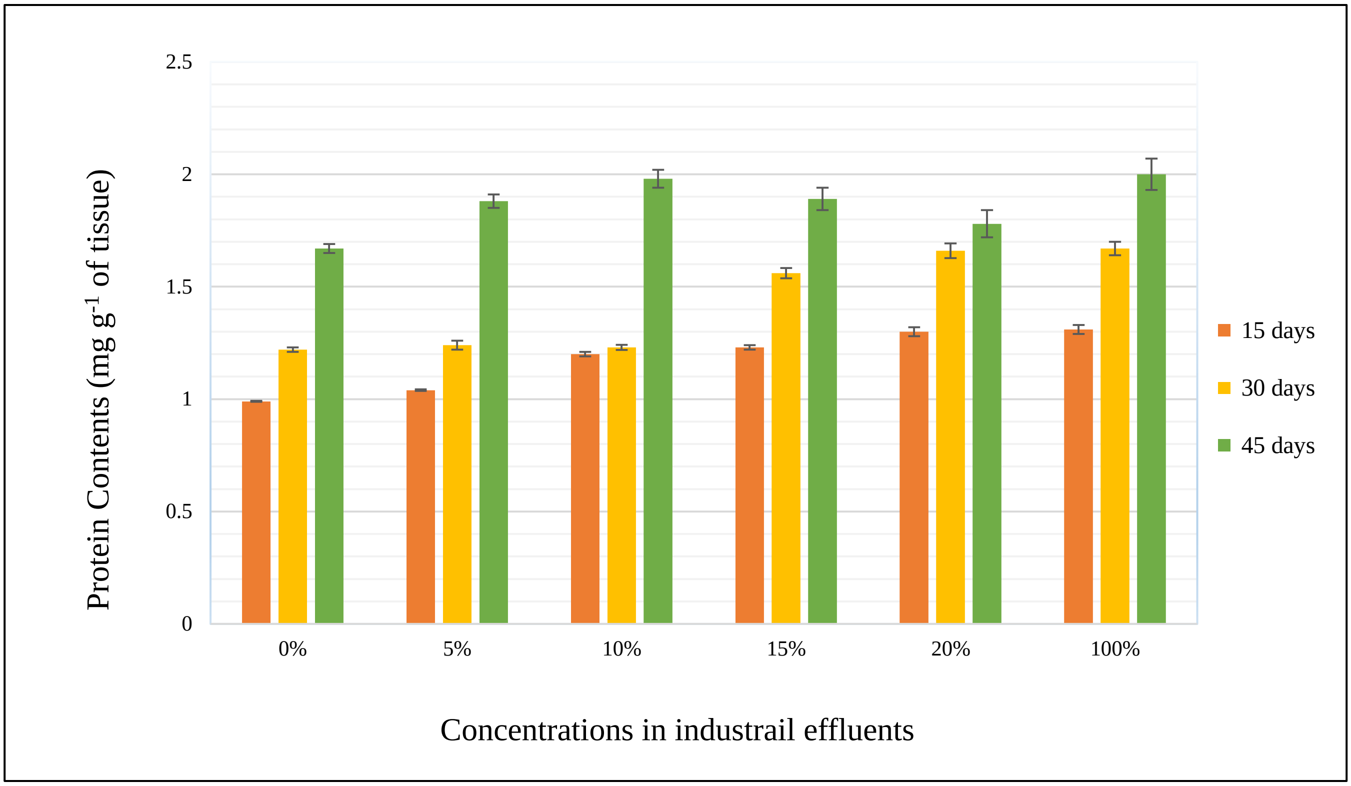

Fig. 3: Determination of Protein content in leaves of 45 days old plants of *Eichhornia crassipes* L. grown in different concentrations of industrial effluents.

DISCUSSION

The contamination of the aquatic ecosystem is considered to be an extreme environmental issue that causes serious environmental problems. Soeprobowati *et al*. (2016) evaluated that this pollution of aquatic ecosystem becomes problematic leading to worldwide issues like eutrophication by degrading the water quality. The major cause of contamination is due to various types of pollutants that enter into the aquatic body. There are various sources of pollution that includes: over population, heavy industrialization, urbanization, disposal of untreated domestic waste, immoderate use of various pesticides and fertilization in the agricultural crops (Haseena *et al*., 2017). The aquatic ecosystem is heavily loaded with metals content that comes from industrial waste, emissions from vehicles, domestic waste, atmospheric deposition, and several others ways (Wei & Yang, 2010). Industrial waste water excretes into the water bodies is one of the main sources of environmental pollution (Kaur *et al*., 2010). The results of present study revealed that all physico-chemical parameters were high in 100% effluent concentrations. The pH of the effluent concentrations is found to be lowest in 0% and highest in 100%. The pH was increased with increase in concentration of effluents that show alkaline nature. These results are according to the Chockalingam *et al*. (2019) who found that the pH of the collected samples (textile industry) is found to be lowest with 8.1 and highest with 8.6 that show a slightly alkaline nature. The alkalinity can be due to the use of various varieties of dyes in the process. The TDS value was found to be highest in industrial effluents than in the control. These results were similar to the Chockalingam *et al*. (2019) who stated that the effluent showed a high level of TDS values when he compared two different samples. This higher level may be due to the discharge of toxic chemical used during different processes in the textile industries and released into the rivers that cause water pollution. This high value of TDS caused salinity problems in the nearby surroundings (Kolhe & Pawar, 2011), and posed a negative influences on aquatic life and agricultural aspects (Roy *et al*., 2010; Kant, 2012).

The electrical conductivity (EC) was found to be maximum in effluents and increased with increase the concentration of wastewater. Similar results were obtained by Aniyikaiye *et al*. (2019). Maximum chlorides were found to be in 100%

concentration and exceeded the tolerance limit by the plants. Finding of this study confirmed the results of Sathyaseelan *et al*. (2015). The COD and BOD was found to be highest in industrial water, and increased with increasing the concentrations of effluents. These findings of industrial effluent are in line with the results of Chockalingam *et al*. (2019) who stated that the standard permissible limit of BOD was 100 mg L^{-1}.

The morphological attributes recorded in our research indicated that plants showed maximum growth in 0% and minimum growth in 100% effluents concentration. The effluents decreased the shoot length of the plants. Jian *et al*. (2019) found the similar results in their research. The chlorophyll content was high in control plants as compared to the other concentrations, as it was decreased with increasing concentrations of the effluents. Houri *et al*. (2019) too stated that the chlorophyll was higher in control than the other sites. A reduction in chlorophyll content results in the inhibition of chlorophyll synthesis or its destruction or replacement of Mg ions (Chandra *et al*., 2009). In control the chlorophyll concentration increased throughout the experiment but in industrial effluents the chlorophyll content was decreased. These results are also in line with Bhattacharya & Banerjee, (2010) who found that the plants grown on various concentrations of sludge showed less chlorophyll content than in the control after 90 days of plant growth. The decrease in chlorophyll content might be due to the reason that heavy metals can substitute the central Mg ion or can inhibit chlorophyll synthesis by inhibiting chlorophyll synthesizing enzyme activity (Manios *et al*., 2003). Decrease in shoot chlorophyll content with time was also due to the production of non-chlorophyllous tissues by shoots. However, it appears that the decrease in chlorophyll concentration was not too much to affect the plant growth.

The concentrations of the heavy metals in the industrial effluents were analyzed and these were in the order of Cr > Cd > Pb and increased with the increasing concentrations of the effluents. The amount of Cr was found to be the highest in the industrial effluents. These results were similar to those of Nazir *et al*. (2015) who demonstrated that the concentrations of the metals in the samples analyzed were in the order of Na > K > Mg > Pb > Cd > Zn. The amount of heavy metals in the samples increased the permissible limits.

The main focus of this study was the heavy metals accumulation capacity of *Eichhornia crassipes* for phytoremediation process. The accumulation of heavy metals was higher in the roots of water hyacinth than in the leaves. The high accumulation of metals in the roots of *Eichhornia crassipes* suggested that the plant was a good hyper accumulator of Cr, Cd, Pb and other pollutants. The highest amount of metals was observed in roots and shoots of *Eichhornia crassipes* in 100% concentration of industrial effluent after 45 days of the experiment. These results showed that the *Eichhornia crassipes* accumulated higher concentrations of Cr, Cu and Pb than normal limits in the roots (i.e., control). These findings confirmed the results by Mishra *et al*., 2009; and Irshad *et al*., 2015. According to them, the concentrations of heavy metals vary significantly with various plant species. The amount of metals among various plant species was in the order of Fe > Zn > Cr > Pb > Ni > Cd > As. The amount of metals in both roots and shoots was found to be relatively higher than the control plants.

The concentration of the protein was analyzed in the plants of *Eichhornia crassipes* after 45 days of the experiment. The amount of protein was found to be highest in 100% concentrations of the effluents. The minimum amount of protein was observed in the control. Bhattacharya & Banerjee, (2010) had found similar results from their research. So at the end, it could be concluded that untreated heavy metals with effluents that are discharged from the industries into the water bodies cause serious environmental pollution. To reduce the environmental or water pollution, phytoremediation technique could be effectively used to extract heavy metals from wastewater.

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