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EFFECT OF *SPIRODELA POLYRHIZA* ON PHYSIO-CHEMICAL CHANGES IN INDUSTRIAL WASTEWATER

TAMSEL AHAMMED[1], SHAHRIAR ISLAM[2], SABRINA ZAMAN[1], MD. RUHUL AMIN[1], MD. MOSTAFA KAMAL[1] AND MD. OMAR FARUQUE*[1]

[1]Department of Nutrition and Food Technology, Faculty of Applied Science and Technology, Jashore University of Science and Technology, Jashore 7408, Bangladesh
[2]School of Food Science, Sichuan Agriculture University, Ya'an 625014, China

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ABSTRACT

The biodiversity has experienced a significant increase in heavy metal contamination, which has a direct impact on aquatic environments. Industrial wastewater, municipal waste, burning fossil fuels, mining, and pesticides are the main sources of pollution. Due to its ability to produce a significant amount of biomass and its high level of stress tolerance, *Spirodela polyrhiza* (*S. polyrhiza*) is known as a phytoremediation aquatic plant. Because of their rapid development, simplicity in harvesting, and potential to remove heavy metals from wastewater *S. polyrhiza* could be an important tool for the treatment of industrial wastewater. This research analyzes how incorporating *S. polyrhiza* into industrial effluent can improve its physiochemical characteristics, like pH, Chemical oxygen demand (COD), Chlorides, total hardness, sulphates, total dissolved solids (TDS), Cu, Ni, Fe, Zn, Pb, and Ca. In this experiment industrial wastewater was treated using *S. polyrhiza* for 45 days on the basis of the depth of water (6", 12", and 18"). From the experimental study after D45 color changed brownish to light brownish. After D45 Chlorides level (mg/l) decreased 295.50±17.73 to 251.20±16.007. The initial COD value (mg/l) of the wastewater was 252.330±6.78 and after 45 days of treatment, the value reduced to 93.87±1. The total hardness (mg/l) of the wastewater was reduced from 1862.8±43.9 to 1223.9±50.93 after treatment. On the other hand TDS value (mg/l) increased from 583.6±3.9 to 815.3±15.7 after the treatment. After 45 days of treatment with *S. polyrhiza* the copper level (mg/l) was reduced from 0.170±0.020 to 0.098±0.032. Similarly, the levels (mg/l) of Pb, Fe, Zn, and Ni were reduced from 0.222±0.029 to 0.061±0.035, 10.306±1.429 to 0.596±0.110, 0.168±0.055 to 0.084±0.023 and 0.150±0.029 to 0.078±0.018 respectively. The findings indicate that *S. polyrhiza* might with stand heavy metals and could be a feasible option for the phytoremediation of physiochemical contaminants and heavy metals derived from industrial wastewater.

KEY WORDS : Water treatment, Heavy metals, Industrial waste, Phytoremediation, *Spirodela polyrhiza.*

INTRODUCTION

Water is indeed a necessary resource for agriculture, people, and companies all over the world (Yadav *et al.,* 2017). Water pollution makes it difficult for people to access fresh water, which is one of the biggest issues facing the world's population today (Grant and Sundby, 2002). Excessive water pollution is caused by the direct discharge of industrial waste into the water from industries (Bhatt *et al.,* 2014). Water pollution has many causal factors, and among them is direct heavy metal emissions in the water from industries and other sources (Ray *et al.,* 2015). Heavy metals are abundant both in nature and in toxic chemicals that are emitted by the industries. A significant issue that directly affects both the individual's health and environmental damage is heavy metal contamination (Hogan, 2012).

(*Associate Professor)

Tenacious and toxins nature, heavy metals pose the greatest threat among all water pollutants (Cui and Cheng, 2015). Since 1900, with the rapid development of industries, and heavy metal contamination has grown quickly (Tiller, 1989). The availability of heavy metals in the water has outright impacts on aquatic ecosystems (Aravind *et al.*, 2015). Metropolitan wastes, tars combustion, ammonium nitrate, domestic and industrial effluents, petroleum ores, and pesticide uses in agriculture farms are the principal components of heavy metal contamination (Kabata, 2010; Prabhat, 2008). A class of trace metals known as "heavy metals" includes both metals and alkaline earth metals like arsenic (As), tin (Sn), chromium (Cr), zinc (Zn), manganese (Mg), cobalt (Co), iron (Fe), lead (Pb), mercury (Hg), cadmium (Cd), nickel (Ni), and copper (Cu) (Salem *et al.,* 2000). According to multiple responses, toxicity from heavy metals is now considered serious environmental provocation (WHO, 1996), because of the negative consequences have on plants, wildlife, and soil degradation (Kim *et al.,* 2019). Plant tissues are severely impacted by toxicants and surplus amount of toxicant additionally reduces the photosynthetic rate, growth, and germination rate of plants (Pourrut *et al.,* 2011; Singh *et al.,* 2010). Ecological toxins are produced by excessive concentrations of heavy metals and which effected in both hydrophytes as well as terraneous ecosystems (Guilizzoni, 1991; Påhlsson, 1989). There are multiple methods such as exchange of ions (Smara *et al.,* 2007) electrolysis, ultrafiltration (Aliane *et al.,* 2001), nano-filtration (Mannan *et al.,* 2018; Raj *et al.,* 2020), reverse osmosis (Feng *et al.,* 2007)**,** membrane filtration, bio-sorption process (Kumari *et al.,* 2006; Tiravanti *et al.,* 1997), chemical precipitation, and flotation (Kurniawan *et al.,* 2006; Sekomo *et al.,* 2012) are still in use today to remove metal ions from groundwater. Each of these options has some benefits and usage restrictions (Miretzky *et al.,* 2004). Finding the right technique is extremely challenging. Firstly, though not all are inexpensive, several demand expert labor and lack of technological reliability, and difficult to keep maintain water quality. Secondly, they cause additional toxic elements and environmental pollution (Cohen, 2006). Between all of them, the use of phytoremediation methods has gained popularity (Ray *et al.,* 2015). It is very effective to use aquatic plants and also the microbes that live on them to remove heavy metal ions from the water. This is regarded as a fresh, economical, and environmentally benign technology (Hannink *et al.,* 2001). In order to carry out heavy metals and other pollutants from both water and soil, numerous species of hyper accumulator plants were recognized, and several of them remain in under researchable action. Numerous macrophyte plants such as duckweed (Singh and Malaviya, 2019), Azolla species (Bhatt *et al.,* 2014), *Eichhornia crassipes* (Eid *et al.,* 2021), *Hydrilla verticillata*, and *Pistia stratiotes* (Venkateswarlu *et al.,* 2019) are indeed possible effective plant species for heavy metals phytoextraction. Duckweeds are a variety of small, free-floating aquatic plant species that can be identified throughout the globe. They are primarily spread by vegetative budding on thallus that resembles leaves, or "fronds" (Landolt and Kandeler, 1986; Yadav *et al.,* 2017). Duckweeds have a fantastic protective mechanism against tough conditions, like cold temperature (Oláh *et al.,* 2014). Specificity to pollutants is a requirement for species of plants that aim to eliminate heavy metals from waste water (Tong *et al.,* 2004). In order to combat and adapt to heavy metals, species with promising prospects for phytoremediation exhibit a wide range of tactics (Singh and Malaviya, 2019; Tanwir *et al.,* 2019). Great duckweed, also known as *Spirodela polyrhiza L.* is a widespread plant community component of groundwater found throughout the world. It frequently covers the whole surface of the water of drainages and other standing bodies of water near farming activities (Ziegler *et al.,* 2015). Growing in importance as model plant for both theoretical as well as practical study (Acosta *et al.,* 2021). Due to a number of benefits like increasing efficiency and resistance to stresses caused by heavy metals, it occupies a unique position (Olguín *et al.,* 2005). The use of plants to remove pollutants has gained prominence, and it has been shown that a variety of plants are successfully applied in handling waste water treatment. The current investigation was carried to find out the impact of *S. polyrhiza* on industrial waste water as a result of its possibility to remove contaminants or pollutants (Bhatt *et al.,* 2014). This investigation seeks to figure out how *S. polyrhiza* affects the physical and chemical characteristics of industrial wastewater.

METHODOLOGY MATERIALS

Sample collection and experimental design

Duckweeds (*Spirodela polyrhiza*) were collected from Ambottola in front of the Jashore University of

Science and Technology campus and the collected *S. polyrhiza* sample are three times washed out with the supply of drinking water to eliminate pollutants and maintained in the space for 1 day to regulate the atmosphere. *S. polyrhiza* used in this investigation were collected from a freshwater pond (23°13′53.6"N 89°07′48.2"E), in Jashore district, Bangladesh. Industrial waste water sample was collected from Monghla port area (pasur river). Monghla is one of the largest industrial area and a large source of waste water in Bangladesh. Samples were collected from three different places S_1 (22° 28' 22.8"N, 89°35′54.3"E), S_2 (22°28′23.4"N 89°35′53.5"E) and S_3 (22°28′24.4"N 89°35′54.8"E) in the same river (pasur river). S_2 and S_3 was collected from 150m and 300m way from S_1 respectively. Each sample divided into three containers according to the depth of water container 6 inch, 12 inch and 18 inch respectively. Three sample take place into nine containers. Our water treatment facility continued for 45 days.

Sampling Site Selection

The most important criteria for selecting industrial waste water sampling sites is the major source of the industrial waste from the industries and city residents and convey with the different sources.

Laboratory experiment

The experiment was conducted in the laboratory of Nutrition and Food Technology Department and CSIRL (Centre for Sophisticated Instrument and Research Laboratory) laboratory at Jashore University of Science and Technology, Jashore, Bangladesh using analytical grade reagents. At first initially collected waste water samples were taken at the laboratory of Nutrition & Food Technology Department. All the water samples were prepared here for the test. After preparing the samples, they were taken to CSIRL Lab of Jashore university of Science and Technology to determine heavy metal and mineral concentration. pH, Chemical Oxygen Demand, Total Dissolved Solid (TDS), Total hardness, Sulphates, and Chlorides were determined in the laboratory of Nutrition & Food Technology Department at JUST. The assessment was conducted in accordance with the standard protocol of American Public Health Association, 1998 of water and wastewater analysis (American Public Health Association, 1998).

Chloride determination procedure

Pipette out 20 ml of field sample into conical flax which contains acetic acid and potassium iodide. Mixed properly with a stirring rod and then added 2 drops of potassium chromate indicator. Titration with 0.025N silver nitrate until the color change to brick red. The titration result was recorded and the results were calculated (American Public Health Association, 1998).

Specimen calculation

Where,
The volume of silver nitrate for the sample, V_S= ml
The volume of silver nitrate for blank, V_B= ml
The volume of the sample= 20 ml
The equivalent weight of chloride= 35.45

$$\text{Chlorides (mg/l)} = \frac{(VS\text{–}VB) \times \text{Normality} \times 35.45 \times 1000}{\text{Volume of sample}}$$

Sulphates determination procedure

100ml of wastewater sample was taken in an Erlenmeyer flask (250 ml), add buffer solution (20ml), and mixed properly with a stirrer rod. 10g of $BaCl_2$ crystals were added and mixed with the solution properly for 1 minute. After that, barium sulfate turbidity was assessed after five minutes by adding the solution to a photometer's absorption cell. Sulphate concentration was calculated by comparing turbidity readings to a calibration curve created using SO4 standards (American Public Health Association, 1998).

pH determination procedure

pH was determined by a digital pH meter. pH meter is set on a flat surface and electrode of pH meter is dipped in a buffer solution. Then the pH meter and the electrode are washed by dipping into distilled solution. The electrode is carefully wiped with tissue paper and dipped into the sample and measured the pH meter reading (American Public Health Association, 1998).

COD determination procedure

2.5 ml of waste water sample fill into a tube and 2.5 ml distill water fill into another tube.1.5ml of $K_2Cr_2O_7$ and 3.5 ml of H_2SO_4 reagentadd both tube and mixed properly. Tightly close the tubes and kept in COD digestor at 150 ^{0}C for 2h. After cooling at room temperature transfer to the conical flask. Fill the burette with freshly prepared Ferrous Ammonium Sulphates (FAS). Add 2 drops of ferric indicator and continue the titration till the color change to reddish brown (American Public Health Association, 1998)

Calculation

Where,
A= Volume of FAS used for blank (ml)
B= Volume of FAS used for sample (ml)
M= Molarity of FAS
8000= Mille equivalent weight of Oxygen (g)*1000 ml/l

$$\text{COD (mg } O_2\text{/l)} = \frac{(A-B)*M*8000}{Volume\ of\ sample}$$

TDS determination procedure

Switch on the balance and note down the initial dry weight of crucible carefully. Filter out 20 ml of the field water sample using Whattman filter papers and place into the crucible. With filtered water sample place the crucible inside the oven at 103 ^{0}C and wait till evaporation of water sample. Cool to room temperature in dessicator. Note down the final dry weight of the crucible and calculate the value of TDS (American Public Health Association, 1998).

Calculation

Where,
A= weight of evaporating dish + filtrate
B= weight of evaporating dish on its own
TDS = [(A-B)]* 1000]/ml sample

Total hardness determination procedure

Pipette out 20 ml of standard hard water into a conical flask. Add 5 ml of buffer solution and few drops of Eriochrome Black-T. The indicator, which is originally blue color would acquire a wine-red color. Titrate with EDTA solution taken in the burette, till the wine red color changes to blue which is the end point. Let the burette reading of EDTA be V2 ml. Repeat the above titration method for sample hard water instead of standard hard water. Let the burette reading of EDTA be V3 ml (American Public Health Association, 1998).

Calculation

Where,
M2 = Molarity of EDTA
V2 = Volume of EDTA consumed (burette reading)
M3 = Total hardness of sample water
V3 = Volume of sample hard water in conical flask
M2V2 = M3V3

Preparation of waste water for heavy-metal determination

In the laboratory, initially digested the collected industrial waste water samples by adding 10 milliliters of highly diluted nitric acid to 90 ml of collected waste water sample, heating it to 100 ^{0} C, and then adding 10:1 perchloric acid to the mixture. Volume should be reduced to 10 milliliters after digestion. Following a 15-minute period of cooling at room temperature, the samples were then filtered through what-man filter paper with the addition of distill water. Finally, 50 ml extract was made for each sample by adding additional distill water and then transferred into 100ml reagent bottle. After preparing the samples, they were taken to CSIRL Lab (Centre for Sophisticated Instrument and Research Laboratory) of Jashore university of Science and Technology to determine heavy metal and mineral concentration. The variables chosen for the investigation were Iron(Fe), Copper (Cu), Lead (Pb), Zinc (Zn), Calcium(Ca), and Nickel (Ni). Atomic Absorption Spectroscopy (AAS) were used to determine heavy metal and mineral. Iron(Fe), Copper (Cu), Lead (Pb), Zinc (Zn), Calcium(Ca), and Nickel (Ni)concentration were determine by 248.3nm, 327.4nm, 283.3nm, 213.9nm, 422.7nm, and 232nm wavelength. The assessment of wastewater was conducted using American Public Health Association, 1998 standard method for analysis (American Public Health Association, 1998).
Industrial wastewater treatment by *S. polyrrhiza* at several time durations are such regards
D0- Initially collected wastewater not treatment by *S. polyrrhiza*
D45- 45 day treatment by *S. Polyrrhiza*

Statistical analysis

Triplicate analysis was done and the data were presented as mean and SD values. The IBM Statistical Package for Social Science (SPSS) version 25 was used to analyze the data.Percentage, Paired sample t- test, one-way ANOVA were used for data analysis. All statements presented in this study are at the $p < 0.05$ levels with 95% CI level.

RESULTS

A comparison study of the sample's physical parameters, including color and odor, is given in Table 1. Utilizing *S. polyrhiza*, wastewater treatedfor 45 days.

Self-Appearance method are applied to identify the changes of color and odor between the waste water samples D0 to D45 day intervals on the basis of depth of water (6", 12", and 18"). Initially all the

Table 1. Physical characteristics (Color and Odor) of industrial waste-water

Method	Parameters	Depth of water (Inch)	Timeframe	
			Day (0)	Day (45)
Self-Appearance	Color	6	Brownish	Light brownish
		12	Brownish	Light brownish
		18	Brownish	Light brownish
Self-Appearance	Odor	6	unpleasant	unpleasant
		12	unpleasant	unpleasant
		18	unpleasant	unpleasant

sample were brownish color and after 45 days treatment with *S. polyrhiza* it changed to light brownish color. Odor were unchanged before and after treatment.

The physiochemical variables analyzed in this study are pH, COD, Chloride, Sulphates, TDS, Total hardness and heavy metals (Ni, Cu, Ca, Fe, Zn, Pb) comparison study of the data set is given in Table 2. Utilizing *S. polyrhiza*, wastewater treated for 45 days on the basis of depth of water (6", 12", and 18"). From the observation table, it has been demonstrated that the level of pH of initially collected sample (Mean ±SD) were 7.88±0.25, 7.84±0.08, and 7.84±0.08 and after 45 days of treatment the pH level of waste water was reduced to 7.48±0.02, 7.46±0.02, and 7.61±0.20 according to 6", 12" and 18" depth of water respectively. Chloride level (mg/l) after 45 days of treatment was reduced 295.50±20.47 to 247.33±19.63 and 295.50±20.47 to 254.98±17.41 in the 6" and 18" depth of water. Hardness level mg/l at D45 was reduce 1663.83±51.63 to 1276.93±14.83 in 18" depth of water. COD level (mg/l) was reduced after 45 days of treatment 152.33±7.73 to 92.89±2.08, 151.33±8.03 to 94.56±2.19, and 152.83±7.83 to 96.16±0.11 mg/l in 6", 12", and 18" depth of water respectively. However, the Sulphates level (mg/l) after 45 days treatment was reduced in 6" depth of water 19.54±0.39 to 13.63±0.11, in 12" depth of water 19.55±0.42 to 13.75±0.05, and in 18" depth of water 19.57±0.42 to 13.83±0.21 respectively. On the other hand TDS level (mg/l) was increased after 45 days 585.20±5.01 to 806.47±15.27 in 6" depth water and 582.75±4.88 to 824.50±14.94 in 18" depth of water. Chemical (minerals and heavy metals) analysis of industrial waste-water parameters considered in this study was Iron (Fe), Zinc (Zn), Calcium (Ca), Nickel (Ni), Lead (Pb), and Cupper (Cu). Atomic absorption spectrometry (AAS) were used for estimating the amounts of this trace elements. From experimental study observed that Fe level (mg/l) was reduced after 45 days of treatment 10.97±0.20 to 0.67±0.18, 9.97±1.88 to 0.54±0.13, and9.97±1.88 to 0.57±0.046 according to 6", 12", and 18" depth of water respectively. Zn level (mg/l) at D45 was reduced 0.16±0.06 to 0.062±0.016 in 6" depth of water, 0.16±0.065 to 0.083±0.016 in 12" depth of water and 0.172±0.054 to 0.106±0.005 in 18" depth of water. The level (mg/l) of Cu, Pb, and Ni was reduced after 45 days treatment 0.170±0.023 to 0.069±0.043, 0.222±0.033 to 0.032±0.014, and 0.159±0.044 to 0.070±0.012 in 6" depth of water respectively. 0.170±0.023 to 0.106±0.005, 0.220±0.031 to 0.063±0.031, and 0.145±0.026 to 0.080±0.026 in 12" depth of water respectively. Cu, Pb and Ni level (mg/l) was reduced after 45 days of treatment 0.170±0.023 to 0.119±0.019, 0.222±0.033 to 0.0895±0.035 l, and 0.146±0.029 to 0.095±0.013 in 18" depth water respectively. Ca level (mg/l) was reduced after 45 day treatment 24.22±1.65 to 20.46±0.57, 24.23±2.60 to 16.42±3.054, and 24.22±2.66 to 11.24±0.88 in 18", 12", and 6" depth of water respectively.

Table 3. Represented paired sample t-test analysis of industrial waste-water parameters figured out are pH, COD, chloride, sulphates, TDS, and total hardness and heavy metals (Ni, Cu, Ca, Fe, Zn, Pb). In Table 3. It's been noticed that the average (Mean ±SD) level of pH was reduced after 45 days treatment 7.790±0.161 to 7.516±0.126. Pair sample t-test D0 vs. D45 interval of pH, t/p value were 5.035/ 0.534. The average level (mg/l) of COD was reduced after 45 days treatment 252.33±6.78 to 93.87±1.69. Chloride, Sulphates, and Total hardness level (mg/l) was reduced after 45 days of treatments 295.50±17.73 to 251.20±16.007, 19.55±0.35 to 13.73±0.153 and 1862.80±43.92 to 1223.91±50.93 respectively. D0 vs. D45 interval of chloride, sulphates, and total hardness t/p value are 20.770/ 0.000, 49.409/0.000 and 25.992/0.000 respectively. The reduced level (mg/l) of COD, chloride, sulphates, and total hardness after 45 days of treatments was significantly associated $p< 0.05$. The level (mg/l) of TDS was increased after 45 days

Table 2. Changes of physico-chemical properties of industrial waste-water according to depth of water (6", 12",18").

Method	Parameter	Depth of water (Inch)	Time frame			
			Day (0)		Day (45)	
			Mean ±SD	*p*-value	Mean ±SD	*p*-value*
Digital P^H meter	P^H	6	7.8±0.25	0.431	7.48±0.02	0.325
		12	7.84±0.08		7.46±0.02	
		18	7.84±0.08		7.61±0.20	
Titrimetric method	COD (mg/l)	6	152.33±7.73	1	92.89±2.08	0.514
		12	151.33±8.03		94.56±2.19	
		18	152.83±7.83		96.16±0.11	
Titration method	Chloride (mg/l)	6	295.50±20.47	1	247.33±19.63	0.877
		12	295.50±20.47		251.31±17.09	
		18	295.50±20.47		254.98±17.41	
Spectrophotometer	Sulphates (mg/l)	6	19.54±0.39	0.998	13.63±0.11	0.266
		12	19.55±0.42		13.75±0.05	
		18	19.57±0.42		13.83±0.21	
TDS meter	TDS (mg/l)	6	585.20±5.01	0.726	806.47±15.27	0.428
		12	582.73±2.13		815.06±17.05	
		18	582.75±4.88		824.50±14.94	
EDTA Titrimetric method	Total hardness (mg/l)	6	1862.80±50.72	1	1167.07^{A}±32.05	0.002
		12	1862.80±50.72		1268.73^{B}±6.61	
		18	1663.83±51.63		1276.93^{B}±14.83	
AAS	Fe (mg/l)	6	10.97±0.20	0.676	0.671±0.181	0.372
		12	9.97±1.88		0.540±0.130	
		18	9.97±1.88		0.577±0.046	
AAS	Zn (mg/l)	6	0.16±0.06	1	0.062^{A}±0.016	0.043
.		12	0.16±0.065		0.083^{AB}±0.016	
		18	0.172±0.054		0.106^{B}±0.005	
AAS	Ca (mg/l)	6	24.22±2.66	1	11.249^{A}±0.884	0.003
		12	24.23±2.60		16.423^{AB}±3.054	
		18	24.22±1.65		20.463^{B}±0.577	
AAS	Ni (mg/l)	6	0.159±0.044	0.846	0.070±0.012	0.171
		12	0.145±0.026		0.080±0.026	
		18	0.146±0.029		0.095±0.013	
AAS	Pb (mg/l)	6	0.222±0.033	1	0.032±0.014	0.127
		12	0.220±0.031		0.063±0.031	
		18	0.222±0.033		0.089±0.035	
AAS	Cu (mg/l)	6	0.170±0.023	1	0.069±0.043	0.150
		12	0.170±0.023		0.106±0.005	
		18	0.170±0.023		0.119±0.019	

Note: AAS= Atomic absorption spectrometry, **p*-value was calculated using ANOVA test, $p<0.05$ was considered statically significantly.

treatment 583.56±3.85 to 815.34±15.74. The chemical (minerals and heavy metals) paired sample t-test analysis of industrial waste-water parameters considered are Iron, Zinc, Calcium, Nickel, Lead, and Cupper. Assessment of the sample on the basis of depth of water (6", 12", and 18") treatment was carried out 45 days used of *S. polyrhiza*. The information was noted from the table that the average level (mg/l) of Fe reduced after 45 days of treatment was 10.30±1.42 to 0.596±0.110. Pair sample t-test D0 vs. D45 interval of Fe was 9.710±1.411 and t/p value was 20/0.000. The reduced level (mg/l) of Cu, Pb, and Ni was after 45 days treatment of waste water 0.170±0.020 to 0.098±0.032,0.222±0.029 to 0.061±0.035, and 0.150±0.029 to 0.078±0.018 respectively, however t/p value after 45 days treatment was 6.651/0.000, 13.680/0.000 and 5.589/0.001, respectively. The reduced level (mg/l) of both minerals and heavy metals after 45 days of treatments was significantly associated $p< 0.05$. The level (mg/l) of Ca was reduced after 45 days of treatment 24.22±2.30 to

Table 3. Changes of physico-chemical properties of industrial wastewater after 45 days of treatment with *S. Polyrhiza*

Parameters	Day (0)	Day (45)		0 day vs. 45 day
pH (Mean ±SD)	7.79±0.16	7.51±0.12		5.03/0.534
COD (Mean ±SD)	252.33±6.78	93.87±1.69		78.67/0.000
Chlorides (Mean ±SD)	295.50±17.73	251.20±16.007		20.77/0.000
Sulphates (Mean ±SD)	19.55±0.35	13.73±0.15		49.40/0.000
TDS (Mean ±SD)	583.56±3.85	815.34±15.74		-48.61/0.000
Total hardness (Mean ±SD)	1862.80±43.92	1223.91±50.93	t/*p* value*	25.99/0.000
Fe (Mean ±SD)	10.30 1.42	0.59±0.110		20/0.000
Zn (Mean ±SD)	0.168±0.055	0.084±0.023		4.82/0.001
Ca (Mean ±SD)	24.22±2.30	16.04±4.31		4.93/0.001
Ni (Mean ±SD)	0.150±0.029	0.078±0.018		5.58/0.001
Pb (Mean ±SD)	0.222±0.029	0.061±0.035		13.68/0.000
Cu (Mean ±SD)	0.170±0.020	0.098±0.032		6.65/0.000

Note:*p*-value was calculated using Pair sample t-test, $p<0.05$ was considered statically significant.

16.04±4.31. The level (mg/l) of Zn was reduced after 45 days treatment 0.168±0.055 to 0.084±0.023. The level (mg/l) of Pb was reduced after 45 days of treatment 0.222±0.029 to 0.061±0.035.

In Table 4 according to the results, it has been determined that the Percentage increment / decrement physico-chemical properties of industrial wastewater after 45 days of treatment with *S. Polyrhiza* according to depth of water (6", 12", 18"). Among other parameters Cu, Zn, Ni, Ca, Pb, P^H, COD, chloride, sulphates, and total hardness were reduced according to the depth of water 6"> 12"> 18". Cu level (mg/l) was reduced after 45 days of treatments by *S. polyrhiza* 42.16±19.46 %. The level (mg/l) of Zn and Ni was reduced after 45 days of treatment 46.47±20.70%, and 45.48±16.92%. On the other hand the level (mg/l) of Pb was reduced after 45 days 72.46±13.80%. However, on the basis of depth of water 18" depth of water less trend to reduction of physico-chemical properties than 6" and 12" depth water except Fe. The reduced level of pH after 45 days of treatment was 3.48±2.02%. The reduction level (mg/l) of chloride, total hardness, and COD was after 45 days of treatments 14.98±2.01%, 34.25±3.41%, 62.78±0.84% respectively. On the other hand the TDS level (mg/l) of waste water was increased after 45 days of treatment 39.71±2.39%. After treatment of waste water the plant biomass residue increase the TDS level of water. *S. polyrhiza* is favorable for dealing of industrial wastewater and appropriate for reduction of industrial pollutants.

Table 4. Percentage increment/decrement physico-chemical properties of industrial wastewater after 45 days of treatment with *S. Polyrhiza* according to depth of water (6", 12",18").

Depth of waterFactors	6 inch (% of decrement)	12 inch (% of decrement)	18 inch (% of decrement)	Average (% of decrement)
Fe (Mean ±SD)	93.89±1.56	94.42±1.23	94.04±1.35	94.12±1.22
Cu (Mean ±SD)	60.02±23.97	36.88±7.12	29.57±11.47	42.16±19.46
Zn (Mean ±SD)	61.06±9.51	47.28±19.97	31.08±23.44	46.47±20.70
Ni (Mean ±SD)	53.41±15.16	49.44±20.34	33.58±13.08	45.48±16.92
Ca (Mean± SD)	53.00±8.05	31.48±15.61	14.87±9.14	33.12±19.29
Pb (Mean ±SD)	85.35±5.80	71.82±11.30	60.22±11.24	72.46±13.80
P^H(Mean ±SD)	2.57±3.03	4.88±0.85	2.98±1.69	3.48±2.02
COD (Mean ±SD)	63.17±0.60	62.51±0.88	62.65±1.15	62.78±0.84
Chloride (Mean ±SD)	16.32±2.22	14.93±1.77	13.69±1.72	14.98±2.01
Sulphates (Mean ±SD)	30.25±2.02	29.67±1.29	29.28±0.71	29.74±1.32
Total Hardness (Mean ±SD)	37.29 ±3.01	34.05±2.10	31.40±2.62	34.25±3.41
	(% of increment)	(% of increment)	(% of increment)	Average (% of increment)
TDS (Mean ±SD)	38.80±2.01	39.86±2.49	41.47±1.57	39.71±2.39

DISCUSSION

On the basis of depth of water (6", 12", and 18") utilizing *S. polyrhiza*, industrial wastewater was treated for 45 days. Potential capacity of *S. polyrhiza* by examining the wastewater both before and after treatment, the removal of pollutants from the various wastewater concentrations was studied. From study it has been observed that changes of color and odor between the waste water samples on the basis of depth of water (6", 12", and 18"). Initially all the sample were brownish in color and after 45 days treatment with *Spirodela polyrhiza* color was changed to light brownish. Odor were unchanged before and after treatment. Same outcome found in another study in India (Yadav *et al.,* 2017). From experimental analysis it's been noticed that the reduced level of pH after 45 days was 7.516±0.126. After 45 days of treatment the reduction level of pH was about 4%. The similar change was also reported in other study (Rajalakshmik, 2010). By secreting substances from their roots and adding organic substances to the waste water, the plant species under study attempt to grow and keep a pH level that is close to neutral, as shown by the decreasing value. The reduction level of COD was after 45 days of treatment 93.876±1.692. Related studies gave same decrease 93% (Deval *et al.,* 2012). Decreased in COD was caused by the plant's intake of biological compounds, which possess the ability to detoxify the sample and lower its COD. The capacity of plants to capture various pollutants, which then gather in their body tissue, can be the reason of such reduction. Chloride content at D45 was found as 247.33±19.63mg/l and 254.98±17.41mg/l in the 6" and 18" depth of water. Average reduction level (mg/l) was 251.207±16.007 after D45. By exchanging anions of chloride and cations in water, the ion linked to *S.polyrhiza* roots aids in the assimilation of chloride from sewage. As a result, extending the timeframe causes to decrease the chloride level from the water sample. TDS level (mg/l) at D45 was 806.47±15.27 in 6" depth water, at D45 was 815.06±17.05 in 12" depth water and at D45 was 824.50±14.94 in 18" depth of water. Within the contact of *S.polyrhiza*, TDS level of the sample significantly increased at various retention of times. This could be caused by plant root secretions, additional plant material, and root residues that dissolve in water after a period of time. Total hardness content at D45 was found as 1167.07±32.05 mg/l, 1268.73±6.61mg/l and 1276.93±14.83 in the 6", 12" and 18" depth of water. Average reduction level of total hardness was 1223.914±50.931 after D45. Throughout the interacting with *S. polyrhiza*, the sample's hardness level was decreased over various retention times. High levels of calcium and magnesium salts, which control the overall hardness, were discovered in a sample of industrial wastewater. Since *S. polyrhiza* have the capacity to store dissolved metals, they are additionally employed to eliminate toxic metals from surface waters (Rolli *et al.,* 2007; Tripathi *et al.,* 2003). It is possible that the mechanism for lifting heavy metal inside the roots, in which effective adsorption takes place, is a result of the abundance of carboxylic acid groups at the roots, which causes a substantial cation exchange across cell surface. The same justification was also found in another study (Aravind *et al.,* 2015). In general, phytoremediation eliminates metal ions from water more speedily. Chemical (minerals and heavy metals) analysis of industrial waste-water parameters considered in this study are Iron (Fe), Zinc (Zn), Calcium (Ca), Nickel (Ni), Lead (Pb), and Cupper (Cu) comparison analysis of the data set. Atomic absorption spectrometry (AAS) method were used for estimating the amounts of trace elements. In this study, the reduction level (mg/l) of metal was selected for investigation was Fe> Pb> Zn> Ni> Cu>Ca. Fe and Pb reduction capacity of *S. polyrhiza* was more than 90% and 70%. The similar finding was found another study (Rai *et al.,* 1995). From study it has been observed that Fe level (mg/) of collected sample were 0.671±0.181, 0.540±0.130, and 0.577±0.046 after 45 days of treatment according to 6", 12", and 18" depth of water respectively. Average decrement level of Fe after 45 days were 94.12±1.22%. Zn level (mg/l) at D45 were 0.062±0.016 in 6" depth water, 0.083±0.016 mg/l in 12" depth water and 0.106±0.005 in 18" depth of water. Average decrement level of Zn after 45 days were 46.47±20.70%. Minimum Cu level (mg/l) at D45 were found as 0.069±0.043 in 6" depth of water maximum Cu level at D45 were 0.119±0.019 in 18" depth water. Metal absorption capacity of *S. polyrhiza* was decreased when increased the depth of water. Average decrement level (mg/l) of Cu after 45 days were 42.16±19.46%. Minimum Pb level (mg/l) at D45 were found as 0.032±0.014 in 6" depth of water, 0.063±0.031 mg/l in 12" depth of water and maximum Pb level (mg/l) at D45 were 0.0895±0.035 in 18" depth water respectively. Average decrement level of Pb after 45 days was 72.46±13.80%. A study on *S. polyrhiza* effectiveness in removing of heavy metal pollutants from wastewater. Proportional decrease in heavy metals like Iron (Fe), Lead (Pb), Copper (Cu) and Zinc (Zn) after treatment reduce 96, 95, 79 and 66%, respectively (Loveson A and Sivalingam R, 2015). Again for the same treatment of wetland's heavy metals such as Iron (Fe), Lead (Pb), Copper (Cu) and Zinc (Zn) reduce by 98, 91, 74 and 62%, respectively (Ray *et al.,* 2015). Minimum Ni level (mg/l) at D45 were found as 0.070±0.012 in 6" depth of water 0.106±0.005 and maximum Ni level (mg/l) at D45 were 0.095±0.013 in 18" depth water respectively. Average decrement level of Ni after 45 days were 45.48±16.92% and Ca were 45 33.12±19.29%. Similar result found in another study (Kumar *et al.,* 2016), in which the average reductions level following time interval Nickel (Ni) and Calcium (Ca) were 47% and 49% respectively. The aforementioned findings show that waste water treatment by *S. polyrhiza* is one of the most efficient, economical, and ecologically responsible methods for treating industrial wastewater since it reduces intricate organic matter into simple form. The main goal of this study's is to investigate and assess *S. polyrhiza* ability to purify water from industrial operations. The actions taken to reduce the amount of pollutants from waste water, enhance environmental performance, and preserve as much of the "Ecosystem Balance" as possible using affordable, environmentally friendly methods.

CONCLUSION

Globally, phytoremediation is being encouraged as one of the most inexpensive and effective techniques. In this study potentiality of *S. polyrhiza* for wastewater treatment was investigated. Time frame for this study was 45 days and the depth of water was 6", 12", 18". From experimental analysis it's been determined that the changes have occurred in physico-chemical properties of industrial wastewater by treating of *S. polyrhiza* thereby reduced level of pH after 45 days of treatment was 3.48±2.02%. Chloride, total hardness, COD, and sulphates level of industrial waste water was reduced after 45 days of treatment 14.98±2.01%, 34.25±3.41%, 62.78±0.84%, and 29.74±1.32%. On the other hand TDS level of the sample was increased 39.71±2.39%. Level of heavy metal like Fe, Zn, Ni, Cu, Ca, and Pb was reduced after 45 days of treatments was 94.12±1.22%, 46.47±20.70%, 45.48±16.92%, 42.16±19.46%, 33.12±19.29%, and 72.46±13.80% respectively. From the study it seems, after 45 days of treatment the level of Zn, Ni, Cu, and Ca was reduced less than 50%. On the other hand Pb and Fe level reduction rate was more than 70% and 90% respectively. As a result, *Spirodela polyrhiza* is being utilized to treat industrial wastewater and consider removing toxic metals. Following treatment with this macrophyte, reductions in the majority of water parameters were seen. According to this study, this plant (*Spirodela polyrhiza*) is able to handle wastewater and very effective to extract heavy metals like copper (Cu), zinc (Zn), lead (Pb), calcium (Ca), iron (Fe), and nickel (Ni) with other wastewater contamination.

Conflict of interest

No conflict of interest exists between the authors.

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