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Phytoremediation potential of *Typha latifolia* and water *hyacinth* for removal of heavy metals from industrial wastewater

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A R T I C L E I N F O

Article type:
Research article
Article history:
Received September 2020
Accepted December 2020
April 2021 Issue
Keywords:
Phytoremediation
Industrial wastewater
Heavy Metals
Typha latifolia
Water *Hyacinth*

A B S T R A C T

This study demonstrates the phytoremediation prospective of *Typha latifolia* (TL) and *water Hyacinth* (WH) in industrial wastewater treatment. A comparative study was done to evaluate the percentage removal of turbidity (Tu), electrical conductivity (EC), color (Col), iron (Fe), copper (Cu) and zinc (Zn) from industrial wastewater by using *Typha latifolia* and *water Hyacinth* with respect to uptake time and with different concentrations of industrial wastewater. The experimental results showed that *Typha latifolia* has performed extremely well in removing the 90.03% Tu, 82.31% EC, 95.98% Col, 92.01% Fe, 87.78% Cu and 75.81% Zn from 20% industrial wastewater during 16 days of experimental period. Results also showed that the maximum percentage removal of selected heavy metals by *Typha latifolia* follow the order Fe > Cu > Zn from 20 % industrial wastewater at 16-day experiment. *Water Hyacinth* showed best result for removing 64.15% Tu, 62.19% EC, 50.29% Col, 54.15% Fe and 70.17% Cu from 15% industrial wastewater during 12 days experiment but it has performed extremely well in removing the 85.97% Zn from 20% industrial wastewater after 16 days of experimental period.

Capsule Summary: Current study evaluates phytoremediation prospective of *Typha latifolia* (TL) and water *Hyacinth* (WH) for removal of different heavy metals in industrial wastewater.

Cite This Article As: N. Abbas, M. T. Butt, M. M. Ahmad, F. Deeba and N. Hussain. Phytoremediation potential of *Typha latifolia* and water *hyacinth* for removal of heavy metals from industrial wastewater. Chemistry International 7(2) (2021) 103-111.
https://doi.org/10.5281/zenodo.4559406

INTRODUCTION

Water is fundamental for life and health. Availability of fresh water has become one of the most serious problems today (Grant et al., 2002). In recent years, water pollution problems involving water treatment, especially, the pollution of water resources by heavy metals have become much more thoughtful and anxious by environmental scientists. Industrial activities of current era have brought about in the deposition of high level of several metals worldwide and seriously threatened human life. Pakistan is neither a major polluting country nor a major consumer of natural resources. However, the country's environment is in a state of acute degradation. Environmental issues in Pakistan have resulted from two important concerns, i.e., unhealthy effects of unplanned industrial development and accompanied urbanization. Water pollution has increased to serious proportion due to discharge of unprocessed industrial effluents and municipal sewage into water bodies (Iqbal et al., 2019).

Heavy metals are those elements that exhibit metallic properties such as ductility, conductivity, cation stability, ligand specificity and have an atomic number that is greater than twenty (Ali et al., 2011). Heavy metals ingested through water or food or inhaled, have been widely reported as one of the major causes of cancer, cardiovascular diseases and death (Adams and Yang, 1979; Houston, 2007; Türkdoğan et al., 2003; Zhao et al., 2014). These are toxins that are capable of causing serious damage even at low concentration (Duruibe et al., 2007; Nagajyoti et al., 2010). The currently used method for removal of heavy metals includes solvent extraction (Abdennebi et al., 2017), ion exchange (Zamri et al., 2017), membrane separation (Chitpong and Husson, 2017) reverse osmosis (Al-Alawy and Salih, 2017), chemical precipitation (Azimi et al., 2017) and electro dialysis (Nemati et al., 2017) which are expensive and can be generate toxic sludge which is another serious problem. The limitations faced by physical and chemical treatment technologies could be overcome with the help of phytoremediation.

Phytoremediation is defined as the use of green plants to remove pollutants from the environment or render them innocuous (E, 2005; Poltorak, 2014). It provides an alternative to more aggressive and intrusive conventional form of remediation. It is less expensive, safe, environmental-friendly and green technology for industrial wastewater treatment. A number of aquatic and terrestrial plants, including grasses, herbs, shrubs and trees, have been discovered to have a high tolerance for water and toxicity stress and possess an excellent ability for significant extraction of non-metabolic elements from contaminated environments (Manousaki et al., 2014; Muhammad et al., 2013; Sinha et al., 2007). Part A of (Fig. 1) describes heavy metals hyperaccumulators (HMHs) and part B explains the process of phytoremediation.

Typha latifolia is an aquatic and perpetual plant that propagates through seed and also vegetative by rhizomes (Keane et al., 1999). It belongs to the Cattail family *Typhaceae* (Fernald et al., 1996) that contains two generation and fifty-one species (Christenhusz and Byng, 2016). Cattail belongs to the genus *Typha* that includes thirty-eight species according to the annual checklist if the Catalogue of life (Fragniere et al., 2015). *Typha latifolia* can be established and survive in wastewater (Calheiros et al., 2008) and is also capable of taking up many chemical pollutants and heavy metals (Kumari and Tripathi, 2015). In this way it can be used for phytoremediation of heavy metals from industrial wastewater.

Water *Hyacinth* is a member of pickerel weed family *Pontederiaceae* and genius *Eichhornia* (Wilson et al., 2005). It is fast growing aquatic plant (Wolverton and McDonald, 1979). It can be established and survived in wastewater (Sooknah and Wilkie, 2004). Water *Hyacinth* has been broadly studied for removing environmental pollutants from wastewater (De Casabianca et al., 1995; Jianbo et al., 2008).

Literature indicates that *Typha latifolia* and water *Hyacinth* have not been widely studied in terms of wastewater treatment in comparative form. The major objective of this research work was to develop a new framework of phytoremediation for determination of the heavy metals uptake potential by using *Typha latifolia* and water *Hyacinth* from industrial wastewater.

MATERIAL AND METHODS

Collection of wastewater

Wastewater was collected from Newage cables industries, is situated at 18 km Lahore-Sheikhupura road and stretched over 25 acres of land. The wastewater was collected in plastic bottles and added few drops of nitric acid for preservation and transported to laboratory.

Collection of plants samples

Two different species of plants *Typha latifolia* and water *Hyacinth* were selected for their abundance and widely used for phytoremediation experiments. *Typha latifolia* (TL) was collected from Sem-Nala, Shershah Suri road Gujranwala, Punjab, Pakistan. Collection was down from the site of Sem-Nala, which lies between 32°12'2.21"N latitude and 74°10'0.84"E longitude. Water *Hyacinth* (WH) was collected from local pond of Rajput colony, near Kacha Fattomand road, Gujranwala, Punjab, Pakistan. Collection was down from the site of local pond which lies between 32°11'21.67"N latitude and 74°12'12.26"E longitude. It was packed in polyethene bags and transferred to laboratory for experiments.

Analysis

Analytical grade chemicals and reagents were used in research study. The glass apparatuses used were Pyrex made. Analytical balance (Sartorius, TE214S); pH meter (Cyber Scan pH 500); Conductivity meter (JENWY, 4010); Turbidity meter (HANANA, LP 2000-11); Tintometer (Lovibond, PFXI 995); Atomic Absorption Spectrophotometer (Perkin Elmer, AA400) were used for analysis of water and wastewater before and after treatment.

Experimental setup

Series of experiments were performed at laboratory scale with sufficient amount of sunlight and air by the help of both the plants, *Typha latifolia* and water *Hyacinth*, to investigate the percentage removal of heavy metals and other selected parameters from industrial wastewater with respect to uptake time and concentration. Fresh and healthy plants of *Typha latifolia* and water *Hyacinth* were used by preparing different concentration of industrial wastewater with respect to uptake time.

The experimental setup of experiment 1.1 and 2.1 is shown in (Fig. 2). In this series of experiments, same concentration (20% of Newage cable industrial wastewater) with variation of contact time was used, i.e. from 4 to 16 days. The experiments were designated as water *Hyacinth* WH-0 (control), WH-1 (4 day), WH-2 (8 day), WH-3 (12 day) & WH-4 (16 day), and in terms of *Typha latifolia* TL-0 (control), TL-1 (4 day), TL-2 (8 day), TL-3 (12 day) & TL-4 (16 day).

Experiment 1.2 and 2.2 were set for same period of uptake time (16 day) but with different concentration of industrial wastewater which contain equal volume of industrial wastewater and equal weight of both the species, as for water *Hyacinth* WH-0 (control), WH-5 (5%), WH-6 (10%), WH-7 (15%) & WH-8 (20%) and *Typha latifolia* TL-0 (control), TL-5 (5%), TL-6 (10%), TL-7 (15%) & TL-8 (20%), and shown in (Fig. 2). Distilled water was used to prepare the respective concentrations of industrial wastewater. Distilled water was added daily to each container to compensate for water loss through plants transpiration and evaporation (Kumari and Tripathi, 2015). All the plants were removed from each container according to their respective time duration by adjusting the volume to 2L and recommended the water samples to laboratory for analysis through proper way. Standard stock solutions (1000 ppm) of Fe (III), Cu (II), Zn (II) were prepared by dissolving 4.8405 g of $FeCl_3\ 6H_2O$, 3.9292 g of $CuSO_4\ 5H_2O$ and 2.0845 g of $ZnCl_2$ in 1M HNO_3 up to liter of distilled water in volumetric flasks separately. Calibration standard solutions were prepared from stock solutions according to dilution method by keeping in view the detection limits of instrument for respective metals. Blank solutions were prepared according to same procedure as adopted in samples.

Analysis of wastewater

All the wastewater samples initially (before the experiments) and finally (after the experiments) were analyzed in turbidity meter (for turbidity), lovibond tintometer (for colour) and conductivity meter (for electrical conductivity) according to method (Rump, 1999). Final analysis was down by using control samples as reference samples. For every analysis three readings were taken to obtain the average value.

For metals analysis, digestion of wastewater samples was carried out by taking 100 ml of its volume into digestion flask by adding twenty ml of digestion mixture (67% Nitric acid: 37% Hydrochloric acid in 1:1) and heating on digestion unit until the clear solution was obtained (Azeem, 2009). These digested samples were taken into 100 ml calibrated volumetric flasks and marked up to volume by adding distilled water. Finally, these samples were analyzed in Flame Atomic Absorption Spectrophotometer according to Elmer method (Elmer and Conn, 1982) for metal analysis.

Data analysis

Percentage removal of turbidity was calculated using Eq. 1.

$$\text{Removal of turbidity (\%)} = \frac{Tu_{ini} - Tu_{fin}}{Tu_{ini}} \times 100 \qquad (1)$$

Where, Tu_{ini} = initial turbidity of industrial wastewater, Tu_{fin} = final turbidity of industrial wastewater. Percentage removal of colour was calculated using Eq. 2.

$$\text{Removal of colour (\%)} = \frac{Col_{ini} - Col_{fin}}{Col_{ini}} \times 100 \qquad (2)$$

Where , Col_{ini} = initial colour of industrial wastewater, Col_{fin} = final colour of industrial wastewater. Percentage removal of electrical conductivity (EC) was calculated using Eq. 3.

$$\text{Removal EC (\%)} = \frac{EC_{ini} - EC_{fin}}{EC_{ini}} \times 100 \qquad (3)$$

Where, EC_{ini} = initial electrical conductivity of industrial wastewater, EC_{fin} = final electrical conductivity of industrial wastewater. Percentage removal or removal effeciency of each metals was calculated using Eq. 4 (Cortes-Esquivel et al., 2012).

$$\text{Removal (\%)} = \frac{C_{ini} - C_{fin}}{C_{ini}} \times 100 \qquad (4)$$

Where, C_{ini} = initial concentration of metal in industrial wastewater, C_{fin} = final concentration of metal in industrial wastewater.

Statistical analysis

Statistical analysis was used to compare the results of both the experiments. Paired samples T-test was performed to compare, the mean percentage removal of selected heavy metals obtained from both the experiments with the help of IBM SPSS Statistics 21. Percentage removal of iron, copper and zinc of both experiments were compared with respect to selected effects like effect of uptake time and concentration.

RESULTS AND DISCUSSION

Physico-chemical characteristics of wastewater

Untreated industrial wastewater was used for all of the experiments. Analysis for selected parameters was carried out in laboratory. Analysis for each parameter was performed three times to obtain mean and standard deviation. Following results were obtained when original industrial wastewater was analyzed for selected parameters along with their National Environmental Quality Standard (NEQS) values. The result of pH and electric conductivity were found higher in range as compared to NEQS (Table 1). The values of turbidity and color were also found higher in range.

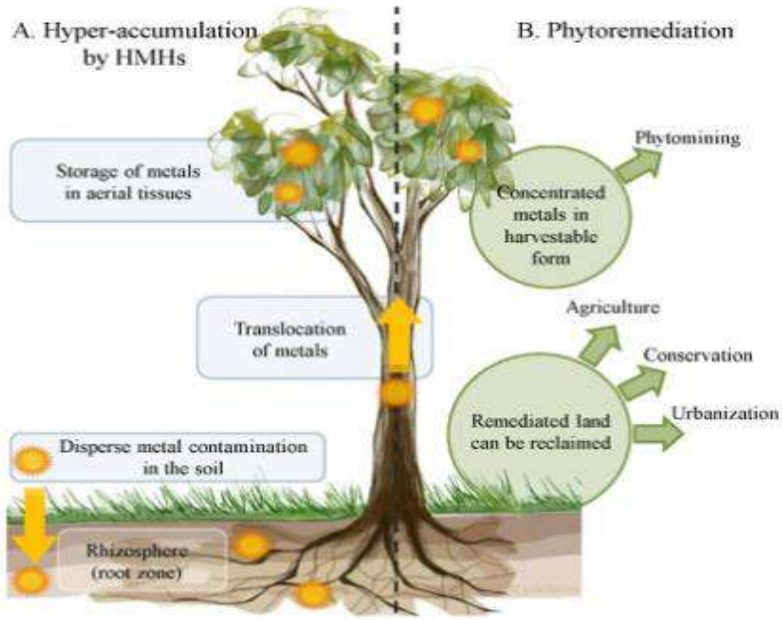

Fig. 1: Part A describes the phenomenon of hyperaccumulation by heavy metals hyperaccumulators (HMHs) and part B explains the process of phytoremediation (Purakayastha and Chhonkar, 2010).

Fig. 2: Water *Hyacinth* and *Typha latifolia* at different wastewater concentration and uptake time

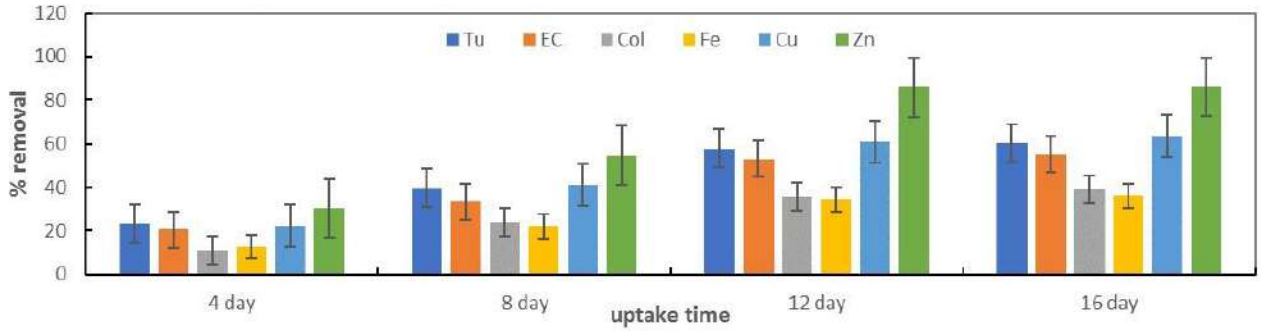

Fig. 3: Percentage removal of heavy metals and other parameters with respect to uptake time by water *Hyacinth*

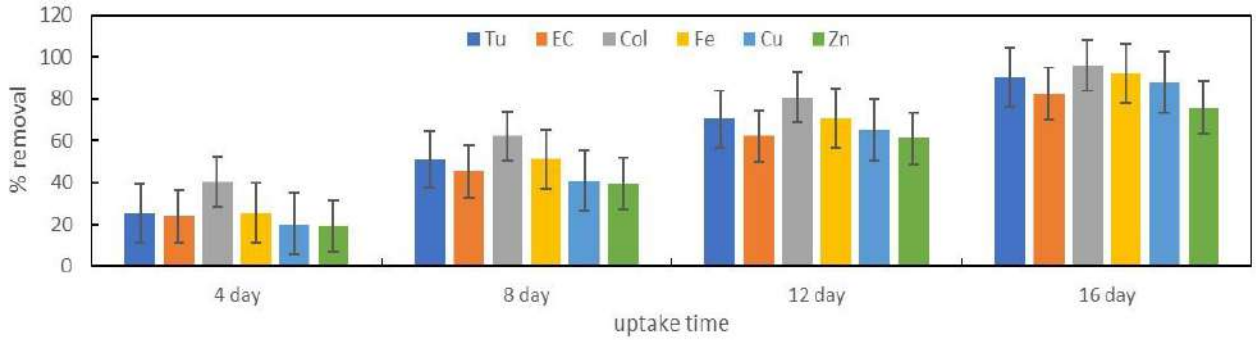

Fig. 4: Influence of uptake on percentage removal of heavy metals and other parameters by *Typha latifolia*

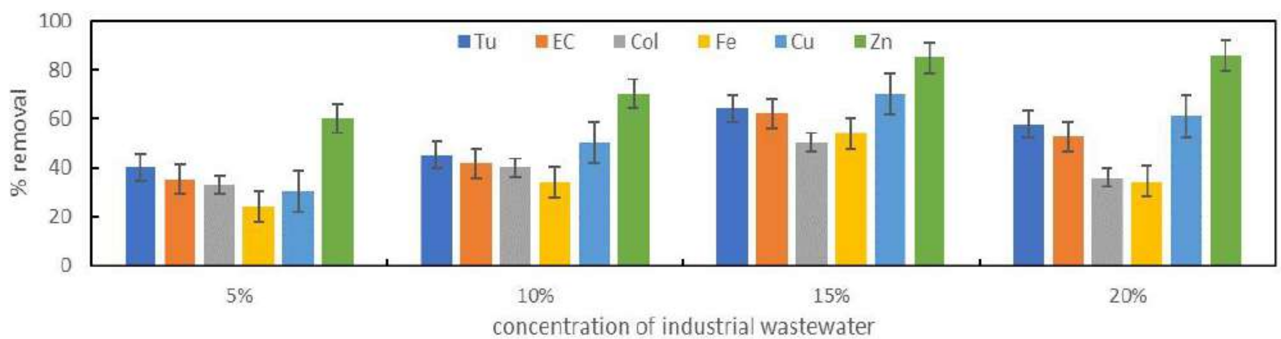

Fig. 5: Influence of concentration on percentage removal of heavy metals and other parameters by water *Hyacinth*

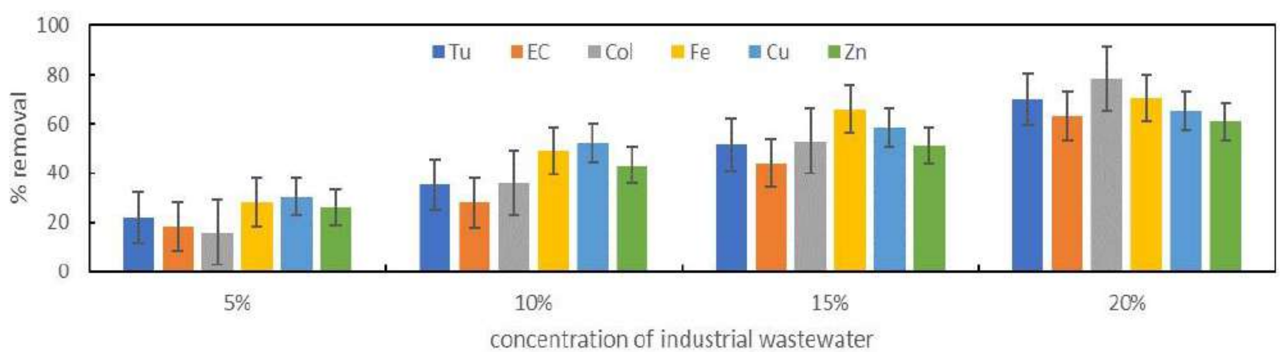

Fig. 7: Influence of concentration on percentage removal of heavy metals and other parameters by water *Hyacinth*

The contamination level of collected wastewater was beyond the safety levels prescribed by the National Environmental Quality Standards (NEQS). The levels of three metals including Fe, Cu and Zn were determined. The study evidenced excessive levels of Cu (8.7246 mg L^{-1}) and Fe (10.5123 mg L^{-1}) in original industrial wastewater that should needed for proper treatment before disposal.

Effect of exposure time on phytoremediation

Theoretically, it was assumed that the rate of percentage removal of heavy metals and other selected parameters would increase as the uptake time increases. From the Fig. 3 and Fig. 4, it has been proven that there was increasing the uptake time, percentage removal of heavy metals and another selected parameter increased. Figure 3 shows that the rate of percentage removal of heavy metals and other selected parameters is high between 4-12 days because after 12 days the condition of water *Hyacinth* was begun to degrade due to accumulation of contaminants. According to Figure 5 the percentage removal rate of heavy metals and other selected parameter lead from 4–16 days because *Typha latifolia* did not degrade due to uptake of contaminants.

From the Figure 3, percentage removal of Tu, EC and Col by water *Hyacinth* follow the order Tu > EC > Col with respect to uptake time. Tu had highest percentage removal up to 60.2% followed up by EC with 55.12% and Col with 38.91% at 16-day experiment. Kulkarni et al. (2007) and Elias et al. (2014) have also reported that water *Hyacinth* show feasible results for reduction of heavy metals and some other parameters of wastewater by phytoremediation process with respect to contact time. It can be also seen in Figure 3 that percentage removal of heavy metals by water *Hyacinth* follow the following order Zn > Cu > Fe with respect to uptake time. Zn had highest percentage removal efficiency up to 85.97% followed up by Cu with 63.71% and Fe with 36.13% at 16 days experiment.

It can be seen in Figure 4 that percentage removal of heavy metals by *Typha latifolia* follow the following order Fe > Cu > Zn with respect to uptake time. Fe had highest percentage removal efficiency up to 92.01% followed up by Cu with 87.78% and Zn with 75.81% at 16 days experiment on the other hand Col. show highest percentage removal up to 95.98% followed by Tu with 93.03% and EC with 82.13% at 16 days experiment. Kumari and Tripathi (2015) and Klink (2017) have explained that by increasing the uptake time in phytoremediation of heavy metals by *Typha latifolia* the reduction of heavy metals in wastewater is increased.

Effect of effluent nature of phytoremediation

From the Figure 5, it can be seen that there was increasing the concentration of industrial wastewater from 5% to 15% with water *Hyacinth* the percentage removal of heavy metals and other selected parameter increased but decreased at 20% wastewater except zinc for 12 days experiment. Saha et al. (2017) and Kumar et al. (2018) have concluded that water *Hyacinth* has proved best as to degrade the water pollutants at low concentration of industrial wastewater. Figure 5, it can also be shown that, percentage removal of selected heavy metals follows the order Zn > Cu > Fe at 20% wastewater for 12 days experiment. Zn had highest percentage removal efficiency up to 85.84% at 20% wastewater followed up by Cu with 70.17% and Fe with 54.15% at 15% wastewater for 12-days experiment on the other hand Tu had highest percentage removal up to 64.15% followed up by EC with 62.19% and Col with 50.29% at 15% wastewater for 12 days experiment.

Typha latifolia shows excellent behavior of heavy metals uptake with respect to different concentration of industrial wastewater. It can be seen from the Figure 6 that there was increasing the concentration of industrial wastewater with *Typha latifolia* the percentage removal of heavy metals and other selected parameter increased and maximum percentage removal of selected heavy metals follows the order Fe > Cu > Zn at 20% wastewater. Hegazy et al. (2011) and Sukumaran *(2013)* have concluded that phytoremediation experiment with *Typha latifolia,* increases the uptake potential of water pollutants with increasing concentration of wastewater. Fe had highest percentage removal efficiency up to 70.39% followed up by Cu with 65.12% and Zn with 60.80% at 20% wastewater on the other hand color had highest percentage removal up to 78.29% followed up by Tu with 69.99% and EC with 63.10% at 20% wastewater.

Statistical analysis

Percentage removal of iron (Fe), copper (Cu) and zinc (Zn) of experiment 1.2 was compared with percentage removal of iron (Fe), copper (Cu) and zinc (Zn) of experiment 2.2 by paired samples t-test (dependent t-test). The result in Table 2 showed that there is some difference in the percentage removal of iron by experiment 1.2 Fe1.2 ($M = 36.7400$, $SD = 12.51693$) and iron by experiment 2.2 Fe2.2 ($M = 53.3625$, $SD = 19.13154$), copper by experiment 1.2 Cu1.2 $M = 52.9325$, $SD = 17.14993$) and copper by experiment 2.2 Cu2.2 ($M = 51.5375$, $SD = 15.09746$), zinc by experiment 1.2 Zn1.2 ($M = 75.2825$, $SD = 12.35801$) and zinc by experiment 2.2 Zn2.2 ($M = 45.3325$, $SD = 14.76131$), but their significance depend upon the probability values.

It can be seen in the Table 3 that there is no significant difference between the percentage removal of iron in experiment 2.2 and experiment 1.2 $t(3) = -2.413$, $p = 0.095$ (2-tailed) which was greater than cut point. In the same way there is no significant difference between the percentage removal of copper in experiment 1.2 and experiment 2.2 $t(3) = 0.392$, $p = 0.721$ (2-tailed) but significant different was found between the percentage removal of zinc in experiment 1.2 and experiment 2.2 $t(3) = 12.925$, $p = 0.001$ (2-tailed). As the p value is less than 0.05

(i.e $p < 0.05$), it can be concluded that there is statistically significant difference between the pairs and p value greater than 0.05 (i.e., $p > 0.05$) there is no statistically significant difference between the pairs (Loftus and Masson, 1994). also showed that the percentage removal of selected heavy metals by water *Hyacinth* follow the order Zn > Cu > Fe from 20% industrial wastewater at 16 days experiment. Therefore, it can also be concluded that water *Hyacinth* was not very effective for iron removal as compared to copper and zinc from industrial wastewater. It is recommended that further research should be needed to optimize phytoremediation process for treatment of wastewater effluent for sustainable development.

Table 1: Analysis of raw industrial wastewater before phytoremediation

Parameters	Value	NEQS (Khan 1998)
pH	2.65±0.07	6-7
EC (mS cm^{-1})	15.49±0.25	1.3
Turbidity (NTU)	974±4	---
Color (Pt-Co $Hazen^{-1}$)	1968±5	---
Fe (mg L^{-1})	10.5123±0.0012	2.0
Cu (mg L^{-1})	8.7246±0.0011	1.0
Zn (mg L^{-1})	1.8115±0.0009	5.0

Table 2: Paired samples statistics on experiments

		Mean	N	Std. deviation	Std. error mean
Pair 1	Fe1.2	36.7400	4	12.51693	6.25846
	Fe2.2	53.3625	4	19.13154	9.56577
Pair 2	Cu1.2	52.9325	4	17.14993	8.57496
	Cu2.2	51.5375	4	15.09746	7.54873
Pair 3	Zn1.2	75.2825	4	12.35801	6.17901
	Zn2.2	45.3325	4	14.76131	7.38065

Table 3: Paired samples test on experiments.

		Mean	Std. Deviation	Std. error mean	T	Df	P Sig. (2-tailed)
Pair 1	Fe1.2 - Fe2.2	-16.623	13.7801	-38.55	-2.413	3	0.095
Pair 2	Cu1.2 - Cu2.2	1.395	7.11182	-9.9215	0.392	3	0.721
Pair 3	Zn1.2 - Zn2.2	29.95	4.63441	22.5756	12.925	3	0.001

CONCLUSIONS

The *Typha latifolia* was found to be efficient in the removal of Turbidity (Tu), Electrical conductivity (EC), Color (Col), iron (Fe) and copper (Cu) from 20% industrial wastewater within 16 days experiment. Results also showed that the maximum percentage removal of selected heavy metals by *Typha latifolia* follow the order Fe > Cu > Zn from 20% industrial wastewater at 16 days experiment. Therefore, it can be concluded that *Typha latifolia* was not very effective for zinc removal as compared to iron and copper from industrial wastewater. The water *Hyacinth* was found to be efficient in percentage removal of zinc (Zn) from 20% industrial wastewater within 16 days experiment but moderate in percentage removal of Turbidity (Tu), Electrical conductivity (EC), Color (Col), iron (Fe) and copper (Cu) from 15% industrial wastewater within 16 days experiment. Results

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