Heavy Metal Bioremediation of Wastewater: An Essential Tool in Green Remediation Technology

ABSTRACT

A group of hydrophytes and cyanobacteria assessed the bioremediation process for tannery wastewater (TWW) that contained heavy metals and other pollutants in this study. TWW has evaluated the effectiveness of the biological treatment and characterized the physiochemical parameters (TDS, EC, DO, and COD). The amount of toxic and trace metals was measured using an atomic absorption spectrophotometer. Four toxic heavy metals were also measured in TWW by this study: Zn (0.33 mg/L), Pb (0.17 mg/L), Cu (1.32 mg/L), and Cr (8.43 mg/L). On the other hand, the pH of the first TWW sample was 7.9. In addition, there are 5150 mg/L, 8840 mg/L, 2.70 mg/L, and 10340 S/cm for EC, TDS, COD, and DO, respectively. Additionally, the experiment showed that Salvinia removed toxic heavy metals Cr (70%) and Pb (80%) the best when compared to the control in TWW. Although biological TWW treatment is a lengthy process, it is an effective and environmentally friendly method for environmental remediation. Therefore, methods of treating TWW by using Salvinia and Spirulina can be regarded as green technologies to eliminate pollutants and create a clean environment for living things.

Keywords: Bioremediation; Cyanobacteria; Tannery wastewater; Hydrophytes; Heavy metal

1. Introduction

Water is an important natural resource that supports a wide range of environmental cycles and is essential to the diversity and abundance of life on Earth. Clean water is crucial for our way of life, economy, and wildlife habitats, ensuring the functioning and flourishing of various sectors like manufacturing, farming, tourism, and energy production. Over the past few decades, human activities like industrialization, urbanization, and unplanned agricultural practices have significantly increased the contaminant loads in water. [1] In the upcoming century, the impact of population growth, industrialization, and global warming will be even greater. By 2050, there will likely be a 20–30% increase in the amount of water needed globally for cities, industry, and agriculture [2]. Population growth, industrialization, and global warming are causing water shortages globally, additionally heavy metals and chlorinated organic compounds polluting water resources and food supplies. Since the industrial revolution, water pollution has become a growing concern for both the public and societal authorities, as well as one of the most alarming problems the world is currently facing is water contamination with growing industrialization, the issue of heavy metal pollution for wastewater discharge [3].Nowadays, heavy metal discharge into the environment is a growing issue due to advancements in technology and their use in various industrial processes [4]. Even at low concentrations, heavy metals and their related compounds pose major health risks because they are extremely toxic, carcinogenic, mutagenic, and teratogenic [5-8].Even in small concentrations, heavy metals and their related compounds pose a significant risk to human health because they are extremely toxic, carcinogenic, mutagenic, and teratogenic [9-11]. [Hence, Reducing or eliminating heavy metal contamination is crucial for preventing environmental pollution and reducing their uptake and accumulation through the food chain. It is reported that the maximum contaminant levels for lead, chromium, cadmium, mercury, and arsenic in water are 0.01, 0.015, 0.1, 0.002, and 0.005, respectively, according to the US Environmental Protection Agency [12-13]. Therefore, environmental life is now seriously at risk from heavy metal pollution because of a large amount of heavy metal contamination in water bodies (ref). As a result, numerous health problems such as lung insufficiency, neurological disorders, bone damage, cancer, teratogenic and embryotoxic effects, and hypertension occur in the human body [14-15] According to research conducted thus far, hydrophytes and microalgae have the potential to be a cost-effective and efficient bio-sorbent for the remediation of heavy metal significantly containing wastewater [16-17].

Over the past few decades, industry and industrial production growth have expanded rapidly in Bangladesh. Although Bangladesh's industries contribute significantly to the country's economy, they also have a negative impact on the environment. In contrast, the leather and textile industry produced and disposed of crore liters of untreated wastewater per year. in this circumstance, Untreated effluents can rapidly alter aquatic ecosystems, significantly impacting fisheries and increasing water body temperature, thereby affecting flora and fauna. In addition, since heavy metals from industries seriously endanger human health, it is imperative to remove toxic metals from industrial wastewater in order to preserve the quality of food and ecological life. However, to solve this problem, a variety of methods can be used, but they are expensive and harmful to the environment. Therefore, according to research, cyanobacteria and hydrophytes may be cost-efficient and useful bio-sorbent for cleaning up heavy metal wastewater. It produces valuable biological materials for the synthesis of bioactive compounds and biofuels and recycles nutrients efficiently. This manuscript explores the use of cyanobacteria and hydrophytes for heavy metal bioremediation, discusses strategies for improvement, and presents potential challenges for a greener environment.

2. Methodology

2.1 The collection of tanning effluent

Ruma Leather Industries Ltd, located at Savar provided the collection of tannery effluent.5L plastic containers were used to collect the wastewater sample for this study from the industry's treatment plant outlet pipes and kept at room temperature. During collection, wastewater was found to be colored and had an obnoxious and unpleasant odor. In the study period, the samples were collected four times throughout the study period.

2.2 Collection of Hydrophytes

we selected two types of locally available free-floating hydrophytes as test species *Salvinia cucullataRoxb*. (Family- Salviniaceae) and *Lemna minor* (Family- Araceae) for this research. *Salvinia cucullata and Lemna minor* were collected from a freshwater pond of Kashiani, Gopalgonj. The hydrophytes were preserved in earthen containers with a depth of 0.3m each and a capacity of 40L water for using two weeks of tap water at normal temperature without any nutrients.

2.3 Collection of Cyanobacteria

The cyanobacteria *Spirulina platensis* was collected from the raceway pond at Applied Botany Laboratories, BCSIR Dhaka Laboratories, Bangladesh.

2.4 Culture of Cyanobacteria

Spirulina platensis was cultured in zarrouk's medium of p^H 9.5 at $25^0C \pm 1^0C$ under hygienic conditions. The culture was gently mixed and exposed to white light, which was produced at a rate of 50 mmol photon/m2/s using cool white fluorescent tubes. The light/dark cycle lasted for fifteen hours. The chemicals for zarrouk's medium (Table 1) and Cyanobacteria sample with culture (Figure -1a and 1b) were provided by the Applied Botany Laboratories, BCSIR Laboratories, Dhaka.The chemicals with the amount are given below.

Table 1: Composition of zarrouk medium

Chemicals	**Amount(g/L)**
NaC1	1.00
$NaNO_3$	2.50
K_2SO_4	1.00
$NaHCO_3$	16.8

K_2HPO_4	0.5g
$MgSO_4.7H_2O$	0.2
$FeSO_4.7H_2O$	0.01
$CaCl_2.2H_2O$	0.04
EDTA	0.08
Micro Nutrient (H_3BO_3, $MnCl_2.4H_2O$, $ZnSO_4.4H_2ONa_2MoO_4$, $CuSO_4$)	1ml

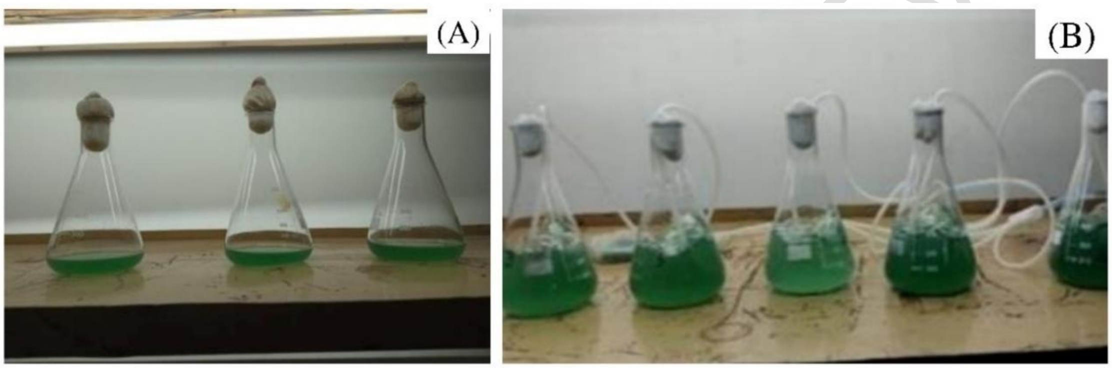

Fig. 1. Cyanobacteria sample (a) and cyanobacteria culture (b)

2.5 Physicochemical analysis of wastewater samples

The collected wastewater was analyzed for various physio-chemical parameters in a soil science lab, BCSIR Dhaka Laboratories, Bangladesh.

2.5.1 pH

Using the HI 9829 Multi-parameter, the water pH level was figured out. After calibration of the pH meter with liquid buffer 7 pH and 9 pH rinse the glass electrode with distilled water, dry it with tissue paper then place it in a wastewater sample and record the pH value.

2.5.2 Total Dissolved Solid (TDS) and Electrical Conductivity (EC)

The wastewater was filtered through Whatman filter paper No.4. Then 50 ml of the filtered sample in 3 sets for treatment and 1 set for control and used glassware. Before starting experimental work all the glassware used during the experiment was washed properly and scrubbed with the nylon brush to remove any adhered salts while the glassware used for heavy melts testing was also washed with 10% HNO_3 to remove any bound metals sample of test water were taken at different times round the course of the experiment was conducted on the 5th, 10th, 15th, 20th, and 25th days to analyze heavy metals and physiochemical parameters. Three replicas are present in each treatment. The experiment continued for 25 days (Fig. 2).

Fig. 2: Experimental setup of hydrophytes-cyanobacteria

2.6 Treatment with hydrophytes

In separate vessels two hydrophytes' species Salvinia and Lemna minor were used in the initial experiment. To eliminate sediments and other contaminants from the regular tap water, all parts of the plants were thoroughly cleaned. Separate glass vessels were used to store 15 grams of Salvinia and 10 grams of Lemna, along with 1 liter of water composed of 70% tannery wastewater and 30% tap water, for treatment.

2.7 Treatment with cyanobacteria

The 2nd experiment setup was conducted with one type of cyanobacteria, *Spirulina platensis* separately. For this treatment, 20 ml *Spirulina platensis* was added into 1L water (70% tannery wastewater + 30% tap water).

2.8 Collaborate treatment with hydrophytes and cyanobacteria

Forthe 3rd set of experiments, two species of hydrophytes, 10g *Salvinia* and 5g *Lemna* were taken along with 15.00 ml *Spirulina platensis*, cyanobacteria in the same treatment vessel.

2.9 Analyzing the level of heavy metals concentrations

To determine the heavy metals Pb, Cr, Zn, Cu, and Cd, we gently evaporated 200 ml of water from each aquarium until it dried. Concentrated nitric acid (HNO3) was used to dissolve the residues in 5 ml. Subsequently, 5–10 drops of hydrogen peroxide (H2O2) were included to make sure the digestion was finished.After the elution of the dried residue with 1 milliliter of HNO3, the concentration of heavy metals was determined using an atomic absorption spectrophotometer (GBC Avanta E, Victoria, Australia; Ser. No. AA7000).

3. Results and Discussion

The study was designed to handle effluent in an environmentally friendly, cost-effective, and simple way.

3.1 Characterizing the characteristics of tannery wastewater

The primary factors that define the properties of tannery wastewater are Total Dissolved Solids (TDS), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Chromate. etc. (Gower, 1980). The permissible limits for collected tannery wastewater samples and their characteristics are explained in Table 1. The sample had a Total Dissolved Solid (TDS) of 5150 mg/L and an Electrical Conductivity (EC) of 10340 <unk> S/cm among the tested parameters. The effluents' pH of 7.9 suggests that they have a slightly alkaline character. The amount of dissolved oxygen (DO) was 2.70 mg/L. It was found that the chemical oxygen demand was 8840 mg/L. Heavy metals including Cr, Cu, Pb, and Zn were found in the tannery effluent, with respective values of 8.43, 0.33, 0.17, and 1.32 mg/L. Other researchers have reported the majority of these findings [18-21]. It was discovered that the TWW sample's TDS, EC, and COD levels were above the threshold. The allowed limit for the concentration of heavy metals, such as Cr, Pb, Cu, and Zn, was exceeded. Nonetheless, the TWW sample's Cd content was found to be within the permitted limit (0.006 mg/L) (Table -2).

Table 2: Compare the parameters of the wastewater sample that was collected with the WHO-approved limit.

Parameters	**Effluent**	**Acceptable Limit**
DO (mg/L)	2.70	4.5
TDS (mg/L)	5150	2100
EC ($\boldsymbol{\mu S}$/cm)	10340	1200
COD (mg/L)	8840	250
pH	7.9	5.5-9
Pb (mg/L)	0.1718	0.1
Cr (mg/L)	8.438	2
Cd (mg/L)	0.0064	2
Cu (mg/L)	0.3312	0.1
Zn (mg/L)	1.3241	1

3.2 Physiochemical parameters of TWW samples

With the industrialization of the country, large volumes of wastewater or effluent are generated daily. A principal factor of water pollution contributes to oxygen demand and nutrient loading in the water bodies, promoting algal biomass and damaging the aquatic ecosystem [22].

This study is focused on assessing the quality level of the tannery effluent that is regularly discharged into municipal drains and ultimately ends up in rivers.

3.2.1The impact of treatments on the DO level of effluent

Dissolved oxygen (DO) is a very important parameter in water analysis. It is an indicator of the physical, chemical, and biological activities of water. The collected TWW sample was treated with *Salvinia* (Sa), *Lemna*(L), and *Spirulina* (Sp) alone or in combination and analyzed for DO, TDS, EC, COD, and pH. The results are presented in Table 3 and Figure (3- 6).DO level was found in the collected TWW sample below the standard limit. More than 4 mg/L is desirable [16] but the simple showed a low amount of DO. DO was found to be 2.70 mg/L in the original sample. The effluent had DO levels of 3.50, 3.63, and 3.75 mg/L after treating Salvinia (Sa), Lemna (L), and Spirulina (Sp). The combined treatment of SSL (Salvinia+ Spirulina + Lemna) resulted in DO levels in the effluent being 4.43 mg/L. On the 25th day, the combined treatment had an impact on the maximum increase in the DO level, which is slightly incorporated with the findings [23].

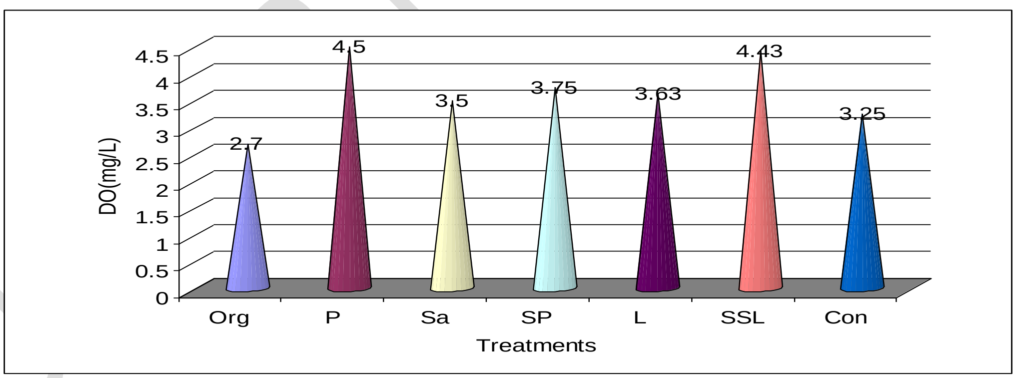

Fig. 3. Different treatments have different variations in Dissolved Oxygen (DO)

(Org: Original sample, P: Permissible limit, Sa: *Salvinia*,Sp: *spirulina*, L: *Lemna*, SSL: (*Salvinia + Spirulina + Lemna*) combined treatment & Con: Control)

3.2.2 ***The impact of treatments on the TDS level of effluent***

In the TWW sample, the TDS level was found to exceed the permissible limit. TDS level was found 5150 mg/L in the original sample. After *Salvinia* (Sa), *Lemna* (L), and *Spirulina* (Sp) treatment, The TDS levels in the effluent were determined to be 3860, 3900, and 3230 mg/L. The TWW effluent had TDS levels of 4290 mg/L after SSL treatment with *Salvinia*, Spirulina, and Lemna. The treatment of Spirulina (Sp) had a significant effect on the maximum decrease in TDS level.

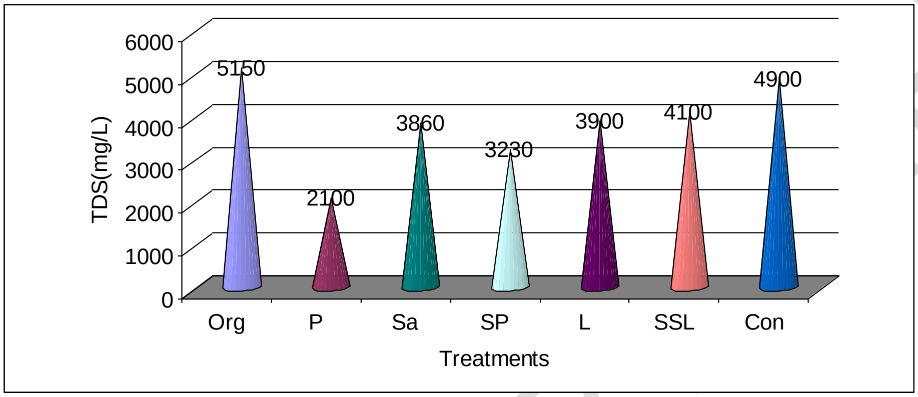

Fig. 4. The total dissolved solid changes based on different treatment

3.2.3 Impact of treatment on the electrical conductivity of effluent

The EC level of the TWW sample was found to exceed the permissible limit. EC was found 10340 µS/cm in the collected TWW sample. The effluent had EC levels of 5,600, 4,800, and 4,660 S/cm after treating Salvinia (Sa), Lemna (L), and Spirulina (Sp).After treating SSL (Salvinia, Spirulina, and Lemna), effluent EC levels were 3290 <unk> S/cm. SSL treatment had an impact on the maximum decrease in the EC level.

3.2.4 Impact of treatments on the COD level of effluent

The COD level of the TWW sample was found 8840mg/L. According to WHO [24] prescribed limit for COD is 250 mg/L. The COD value in the TWW sample is much higher than the permissible level. The effluent showed COD levels of 1600, 800, and 5000 mg/L after using Salvinia (Sa), Lemna (L), and Spirulina (Sp). The effluent had 1200 mg/L COD levels due to the combined treatment of Salvinia, Spirulina, and Lemna. The maximum drop in COD level was influenced by SSL treatment.

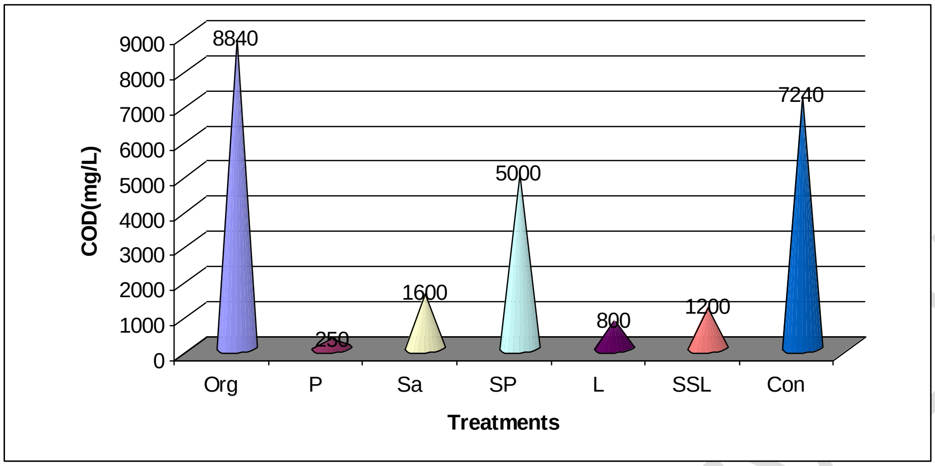

Fig. 5. Different treatments affect the variation of Chemical Oxygen Demand (COD).

3.2.5 The pH of effluent is impacted by treatment:

TheTWW's pH was discovered to be 7.9. The result indicates that the nature of the TWW is slightly alkaline and under the permissible limit of WHO [18]. The effluent's pH levels were determined to be 6.42, 6.49, and 6.51 after treating *Salvinia* (Sa), *Lemna* (L), and *Spirulina* (Sp). The effluent's pH levels were determined to be 6.93 after the combined treatment of SSL (*Salvinia*+ *Spirulina* + *Lemna*). Salvinia treatment resulted in a maximum decrease to its highest level.

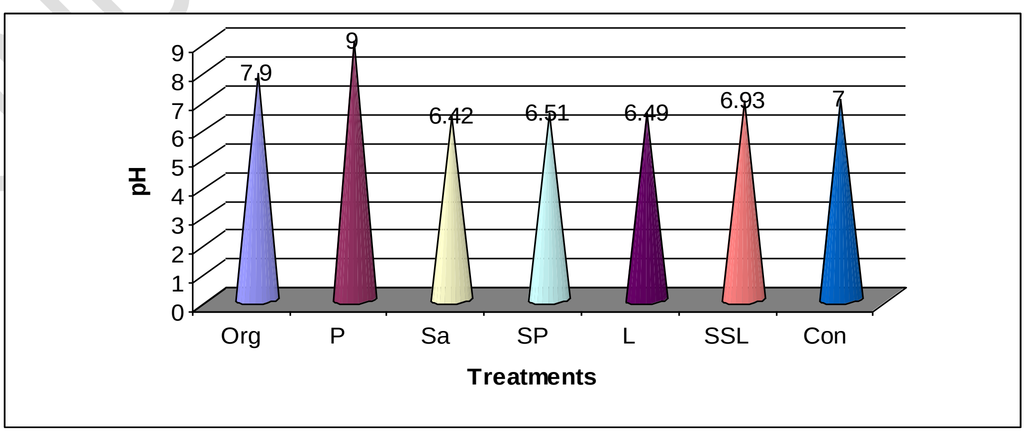

Fig. 6. Changes in pH treatments following various interventions

Table 3: Analysis of the wastewater from the tannery's physiochemical parameters during the experiment

Treatment	**Parameter**	**Unit**	**Zero**	**Day5**	**Day10**	**Day15**	**Day20**	**Day25**
Salvinia	DO	(mg/L)	2.70	2.81	2.89	2.96	3.07	3.50
	TDS	(mg/L)	5150	4800	4530	4300	3900	3860
	EC	(μS/cm)	10340	8900	6800	4660	5000	5600
	COD	(mg/L)	8840	6220	4900	3600	2220	1600
	pH		7.9	7.36	6.97	6.76	6.57	6.42
Spirulinaplatensis	DO	(mg/L)	2.70	2.83	3.08	3.36	3.63	3.75
	TDS	(mg/L)	5150	4500	3340	3660	3560	3230
	EC	(μS/cm)	10340	5420	4980	3800	4300	4660
	COD	(mg/L)	8840	7600	5000	6600	7000	6660
	pH		7.9	7.29	6.95	6.78	6.61	6.51
Lemna	DO	(mg/L)	2.70	2.95	3.07	3.21	3.36	3.63
	TDS	(mg/L)	5150	4900	4440	4330	4200	3900
	EC	(μS/cm)	10340	7800	6000	5600	5300	4800
	COD	(mg/L)	8840	4600	2000	1200	1000	800
	pH		7.9	7.33	6.80	6.72	6.53	6.49
Salvinia + Spriulinaplatensis+ Lemna minor	DO	(mg/L)	2.70	3.26	3.53	3.75	3.96	4.43
	TDS	(mg/L)	5150	4900	4760	4660	4330	4290
	EC	(μS/cm)	10340	5960	4400	4560	3341	3290
	COD	(mg/L)	8840	5000	3800	2500	1600	1200
	pH		7.9	7.30	6.95	6.89	6.90	6.93

3.3 Analysis of Heavy Metals

Heavy metals were analyzed in both treated and untreated effluent, as well as treated plants by acid-digesting all the samples. The estimation of heavy metals was analyzed by Atomic Absorption spectrophotometer (Table 4; Fig 7-10) and after treatment figure 11a,11b, 11c, 11d, and 11e.

3.3.1 Treatment's impact on the concentration of pb in the effluent

Pb level was found in the collected TWW sample above the standard limit. Pb was found 0.1718 mg/L. Following the application of Salvinia (Sa), Lemna (L), and Spirulina (Sp) treatments, the effluent's Pb levels were determined to be 0.124, 0.004, and 0.091 mg/L. Pb levels in the effluent after the combined treatment of SSL (*Salvinia Spirulina + Lemna*) were found to be 0.082 mg/L. So, all the treatments were found effective to reduce Pb level but treatment by *Lemna* was more effective than others.

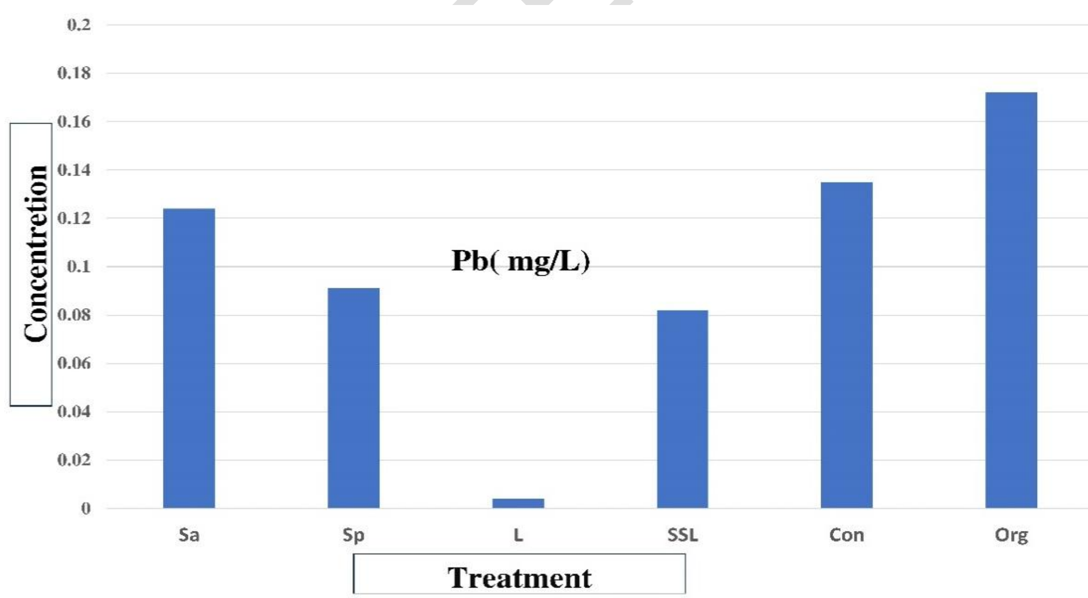

Fig. 7. The concentration of lead (Pb) in the wastewater after treatment with hydrophytes and cyanobacteria.

3.3.2 Treatment's impact on the concentration of Cr in the effluent

Within the TWW sample, the Cr level was not within the standard limit. Cr was discovered at 3.438 mg/L, respectively. Following the treatment of the effluent with Salvinia (Sa), Lemna (L), and Spirulina (Sp), the levels of Cr were determined to be 1.936, 2.544, and 3.602 mg/L, respectively. Following the combined treatment of Salvinia + Spirulina Lemna, the effluent's Cr levels were found to be 3.005 mg/L. The Sa treatment resulted in the greatest drop in the Cr level.

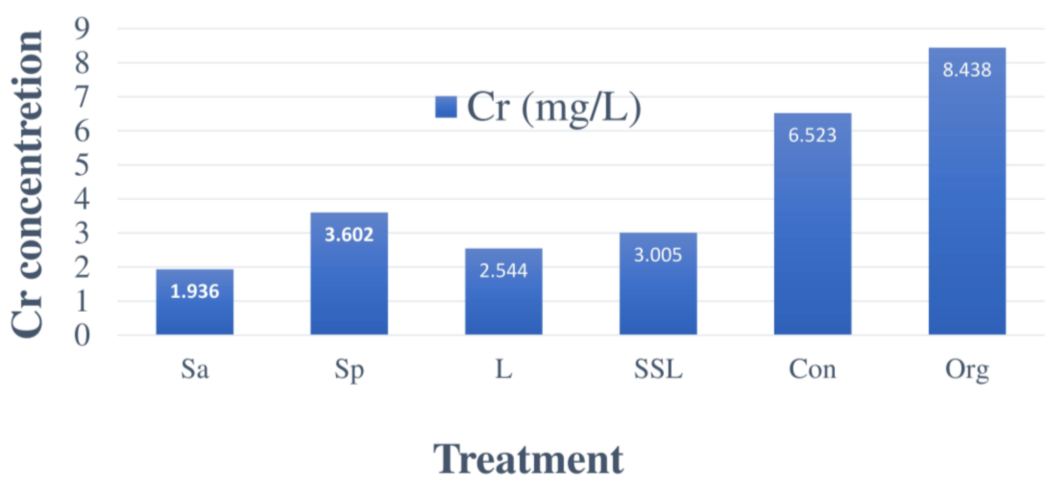

Fig. 8. The concentration of Chromium in the wastewater after treatment with hydrophytes and cyanobacteria.

Table 4: Impact of various cyanobacteria and hydrophytes on various heavy metal parameters in the wastewater effluent from tanneries.

Heavy metal	Sa	Sp	L	SSL	Con
Pb	0.124	0.091	0.004	0.082	0.135
Cr	1.936	3.602	2.544	3.005	6.523
Cd	0.00	0.00	0.00	0.00	0.00
Cu	0.183	0.127	0.146	0.133	0.246

Zn	0.032	0.048	0.97	0.084	1.003

3.3.3 Treatment's effect on the effluent's Cu concentration:

The cu level was above the standard limit in the TWW sample. CU was found at 0.331 mg/L in the sample. After *Salvinia* (Sa), *Lemna* (L), and *Spirulina* (Sp) treatment Cu levels of the effluent were found to be 0.183, 0.146, and 0.127 mg/L respectively. After the combined treatment of Salvinia + Spirulina + Lemna, Cu levels in the effluent were found to be 0.133 mg/L, respectively. The Sp treatment had an impact on the maximum drop in the Cu level.

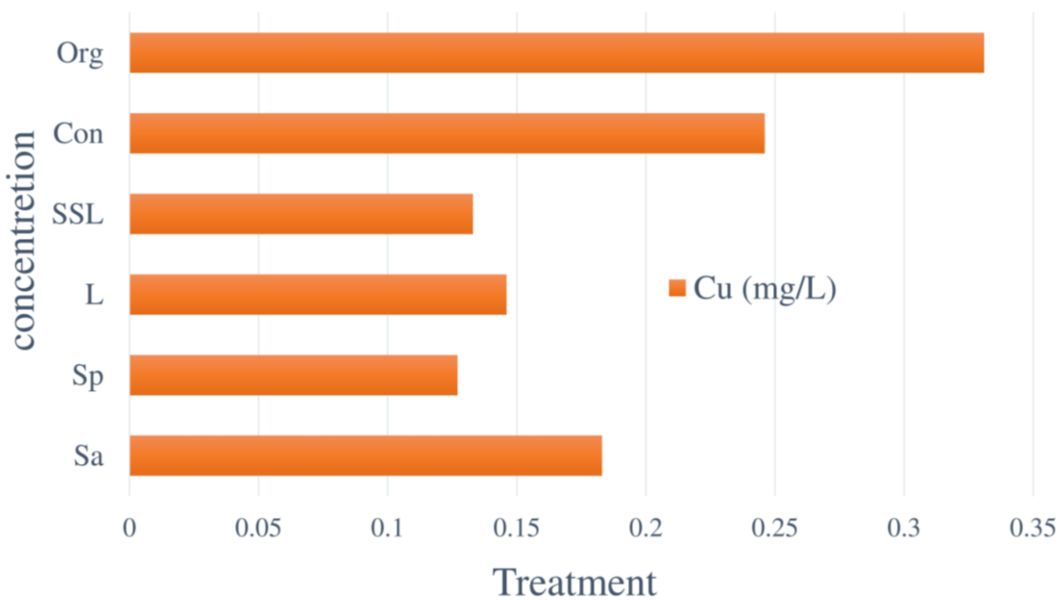

Fig. 9. The concentration of copper in the wastewater after treatment with hydrophytes and cyanobacteria

3.3.4 Treatment's impact on the zinc concentration of the effluent:

Zn was found in the TWW sample more than what was advised. Zn concentrations after treatment with Salvinia (Sa), Lemna (L), and Spirulina (Sp) were found to be 0.032, 0.97, and 0.048 mg/L, respectively, in the effluent. Zn concentrations in the effluent were determined to be

0.084 mg/L after the combination treatment of Salvinia + Spirulina + Lemna, respectively. Salvinia therapy had an impact on the Zn level's maximal decline.

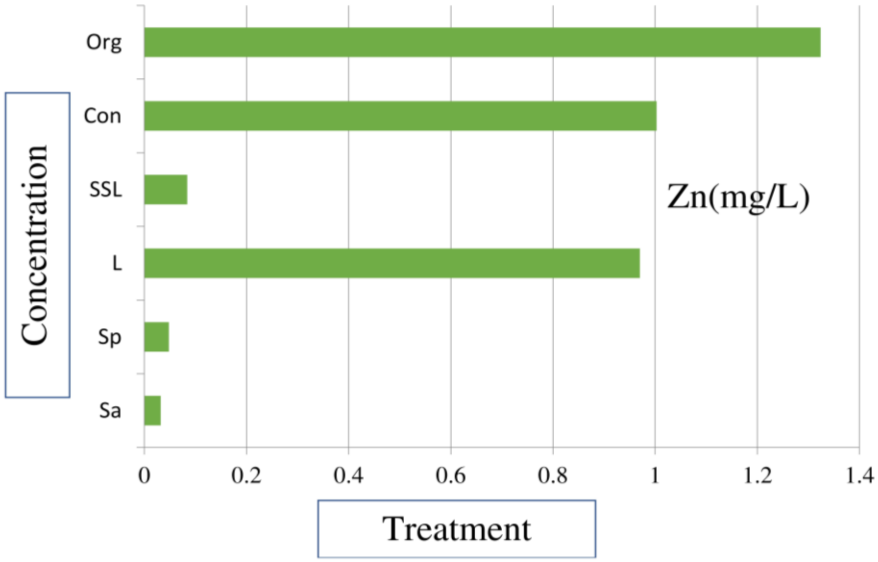

Fig.10. The concentration of zinc in the wastewater after hydrophytes and cyanobacteria treatment

4.Conclusion

Biological remediation of tannery effluent is a cost-effective, environmentally responsible, and easily accessible method that significantly removes heavy metals from the environment. The study suggests that treating industrial wastewater with hydrophytes (*Salvinia cucullata* and *Lemna minor)* and cyanobacteria (Spirulina platensis) can effectively remove pollutants, reduce COD, TDS, and EC, increase Dissolved Oxygen and decrease COD, with the highest DO observe in the combined treatment. In contrast, the sequences of heavy metals removal by *Salvinia cucullata* were found as follows: Zink >Cromium>lead> Copper, by *Lemna minor*, was lead > Chromium > Copper > Zink, by *Spirulina platensis* was Copper > Zink > lead > Chromium, and by a consortium of hydrophytes (Salvinia cucullate and Lemna minor) and cyanobacteria (Spirulina platensis) was lead> Copper > Zink >Cromium. Based on the current research, it can be concluded that after TWW treatment with selected hydrophytes and cyanobacteria alone or in combination, most of the physiochemical parameters of treated water decreased under the permissible limit. The implication of the finding indicates that hydrophytes Salvinia cucullate, Lemna minor, and the cyanobacteria Spirulina can be efficiently utilized as bioremediation agents for the treatment of industrial wastewater. Further investigation is needed to investigation is needed to the optimal conditions for the bioremediation of hydrophytes and cyanobacteria species in resolving Cr and Pb from contaminated sites. Additionally, Further research is required to determine the best ways to re-utilize hydrophytes and cyanobacteria following treatment.

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