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Bioremedial Capacity of Indigenous Hydrophytes and Microalgae of Bangladesh: A Comparative Study on their Potential in Tannery Effluent Treatment

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Authors' contributions

This work was carried out in collaboration among all authors. Author NNH conceptualized the study, performed methodology, wrote, reviewed, edited the original draft and supervised the study. Author MAA investigated the study and did software analysis. Author ASB did data curation and visualization. Author MEAZ performed methodology and searched for resources; Author MZR did data visualization. Author CKR reviewed the manuscript; and Author JLM supervised the study, did project administration and funding acquisition. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to identify the bio-remedial potential of indigenous hydrophytes (*Salvinia cucullata* and *Lemna minor*) and microalgae (*Spirulina platensis*) of Bangladesh for treatment of tannery wastewater disposed in the open environment.

Study Design: The capacity of improving the water quality of the tannery effluent by bioremediation was assessed. The quantitative determination included the comparison of physical characters (pH, TDS, EC, DO, COD) and heavy metal profile (Pb, Cr, Cu, Zn) of the tannery effluent before and after treatment. Effluent treatment was carried out by individual species separately and in combination with all for a total of 25 days.

Place and Duration of Study: Major experiments were carried out at the Applied Botany Laboratory, Dhaka Laboratory, BCSIR, Dhaka, Bangladesh from January 2022 to February 2024. Quantitative estimations were carried out at Soil & Water Laboratory, Dhaka Laboratory, BCSIR, Dhaka, Bangladesh.

Methodology: The tannery effluent was characterized by means of physiochemical parameters and heavy metal concentration prior to the experimental procedure. Four treatment plans were designed, three treatments (TSC, TLM, TSP) using individual species separately and one treatment (TC) using all three species in combination. The treatment continued for 25.0 days. The physiochemical parameters of the treated effluent were measured at 5.0-day intervals and heavy metal conc. Were measured on the 0th, 10th, and 25th days of the experiment. Comparative analysis of the data was utilized to determine useful species for further applied studies in future.

Results: After treatments, a reduction at TDS level down to 3230 from 5150 mg/L, and an EC dropdown to 3290 from 10340 μS/cm was observed. Heavy metals removal capacity of the tested species was found as follows, *S. cucullata*: Zn > Cr >Pb> Cu, *L. minor*: Pb> Cr > Cu > Zn, *S. platensis*: Cu > Zn >Pb> Cr, and the combination treatment: Pb> Cu > Zn > Cr. The observation showed high efficiency of *S. platensis* in TDS reduction and Zn absorption, *L. minor* in COD reduction and Pb absorption, and *S.cucullata* in Zn absorption.

Conclusion: The comparative data suggested the overall improvement of the effluent quality can be achieved by a combined treatment of *L. minor* and *S. platensis,* quickly and cost-efficiently. Further investigation is required for an in-depth understanding of their combined potential. Additionally, investigation will be carried out on their potential in Arsenic (As) removal and nanoparticle production from post-treatment biomass.

Keywords: Bioremediation; tannery effluent; hydrophytes; microalgae; Spirulina; heavy metal; wastewater treatment.

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 20.0 to 30.0 % increase in water needed globally for cities, industry, and agriculture [2]. 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]. Even at low concentrations, heavy metals and their related compounds pose major health risks because they are extremely toxic, carcinogenic, mutagenic, and teratogenic [4-7]. 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 [8-11]. Hence, Reducing or eliminating heavy metal
contamination is crucial for preventing contamination is crucial for environmental pollution and reducing their uptake and accumulation through the food chain. It is reported that the maximum contaminant levels for Pb, Cr, Cd, Hg, and As 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 [14]. 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 biosorbent 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 crores of litres of untreated wastewater per year. The tannery industry belongs to one of the most polluting industrial sectors. Almost every tannery industry uses significant amounts of chemicals in the process of transforming animal hides into leather [18]. The tanning process is almost completely a wet process that consumes a massive amount of water and generates about 90% of the used water as effluent [19]. Tannery effluent carries heavy pollution loads due to a massive presence of highly coloured compounds, sodium chloride and sulphate, various organic and inorganic substances, toxic metallic compounds, different types of tanning materials which are biologically oxidizable and large quantities of putrefying suspended matter. Worldwide, it is estimated that discharged tannery effluent contains 300-400 million tons of heavy metals, solvents, toxic sludge and other wastes, which are dumped into the water each year [19]. Treatment of tannery wastewater is carried out by physical, chemical, biological, or combinations of these methods. 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 [18].

Bioremediation is one of the important pollution control technologies that use some biological systems to degrade various toxic chemicals into less harmful forms. Besides this, bioremediation may be defined in many other ways. According to the Environmental Protection Act (EPA 1976) [20], bioremediation is a "Treatment that uses naturally occurring organisms and plants to break

down dangerous substances into non-toxic substances". Cyanobacteria is found to be an important biological agent for wastewater treatment. *Spirulina* sp. is a cyanobacterium that grows rapidly and contains a detectable level of Mercury and Lead when grown under contaminated conditions, implying that it can take up toxic metals from the environment [21]. The hydrophytes *Lemna* sp., *Salvinia* sp., *Pistia stratiotes* and *Eichhornia crassipes* are a major part of the aquatic ecosystem and show high removal efficiency for Hg, Pd, Zn, Cu, and Mn. They adsorb the contaminants and translocate their roots and shoots from polluted wastewater [22-27] and other several aspects [28-37].

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. However, to solve this problem, a variety of methods can be used, but they are expensive and harmful to the environment. Therefore, 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 Collection of Tannery Effluent

The tannery wastewater (TWW)was collected from Ruma Leather Industries Ltd., located at Savar, Dhaka, Bangladesh. Samples were collected four times throughout the study period from the industry's treatment plant outlet pipes. During collection, wastewater samples were found to be coloured along with an obnoxious and unpleasant odour. 5.0 L plastic air-tight containers were used to collect the samples and preserved at room temperature until used.

2.2 Collection of Hydrophytes

We selected two types of locally available freefloating hydrophytes *Salvinia cucullate* Roxb. (Family- Salviniaceae) and *Lemna minor* (Family-Araceae) for the treatment of TWW. *Salvinia cucullata* and *Lemna minor* were collected from a freshwater pond of Kashiani, Gopalgonj and characterized in the Applied Botany Laboratory, Dhaka Laboratory, BCSIR. The hydrophytes were preserved in tap water at room temperature without any nutrients for two weeks in earthen containers with a depth of 0.3m and a capacity of 40.0 L each.

2.3 Collection of Microalgae

The cyanobacteria *Spirulina platensis* was provided by the Applied Botany Laboratory, Dhaka Laboratory, BCSIR, from its specialized raceway Spirulina culture pond (Fig. 1(A)).

2.4 Culture of Microalgae

Spirulina platensis was cultured in Zarrouk's medium of pH 9.5 at (25 ± 1.0) °C following aseptic conditions. The culture was gently mixed and exposed to white light, which was produced at a rate of 50.0 mmol photon/m² /s using cool white fluorescent tubes (Fig. 1(B)). The light/dark cycle of 14:10 hours was maintained. The reagents for Zarrouk's medium (as mentioned in Table 1) were provided by Applied Botany Laboratory, Dhaka Laboratory, BCSIR.

2.5 Experimental Design for TWW Treatment

The wastewater was filtered through the Whatman filter paper No.4. and samples (TWW: distilled $H_2O = 7:3$ were prepared. The experiment included three different individual treatments of the prepared TWW samples $(T_{SC},$ T_{LM} , T_{SP}), one combined treatment (T_{C}) and one control (C). Each setup contained 1L sample and each treatment was performed in triplicates for 25 days.

2.5.1 Treatment with hydrophytes

Two hydrophytes species *S.cucullata* and *L. minor* were used separately in two sets of experiments $(T_{SC}$ and T_{LM}). To eliminate sediments and other contaminants, all parts of the plants were thoroughly cleaned beforehand. 15.0 g of *S.cucullata* and 10.0 g of *L. minor were* used per liter of water sample for treatment.

2.5.2 Treatment with microalgae

S. platensis was used separately in another set of experiments (TSP)with 20.0 mL of wet *S. platensis* per litre of water sample for treatment.

2.5.3 Combined treatment with hydrophytes and microalgae

For the next set of experiments (T_c) , 10.0 g of *S.cucullata*, and 5.0 g of *L. minor* were taken along with 15.0 mL of wet *S. platensis*, per litre of water sample for treatment.

Table 1. Composition of Zarrouk's medium

Chemicals	Amount (g/L)
NaCl	1.00
NaNO ₃	2.50
K2SO4	1.00
NaHCO ₃	16.8
K2HPO ₄	0.50
MqSO ₄ .7H ₂ O	0.20
FeSO ₄ .7H ₂ O	0.01
CaCl ₂ .2H ₂ O	0.04
EDTA-Na ₂ .2H ₂ O	0.08
A ₅ Micro Nutrient	1.0 L
$(H3BO3, MnCl2.4H2O1)$	
$ZnSO4.4H2O$, $Na2MoO4$,	
CuSO ₄ .5H ₂ O	

2.6 Physicochemical Analysis of TWW Samples

Various physiochemical parameters of the collected TWW samples were analyzed at the Soil Science Laboratory, Dhaka Laboratory, BCSIR, before treatment and compared with the World Health Organization (WHO) standards. Identical tests were performed further for the hydrophyte and microalgae-treated TWW samples for evaluation of the impact of these treatments. Physiochemical analysis of the samples was carried out at 5-day intervals from 0-25 days.

2.6.1 pH

The samples were filtered through Whatman filter paper No.4. Using the Hanna Instruments: HI 9829 multi-parameter, the pH level was measured for the filtered samples. Prior to recording the pH values, the meter was calibrated using buffers of pH 7.0 and pH 9.0.

2.6.2 Total Dissolved Solid (TDS)

The filtered sample was used for measuring the TDS using the Hanna Instruments: HI 9829 multiparameter.

2.6.3 Electrical Conductivity (EC)

The filtered sample was used for measuring the EC using the Hanna Instruments: HI 9829 multiparameter.

2.6.4. Dissolved Oxygen (DO)

The filtered sample was used for measuring the DO using the HACH Instrument: HQ 3 OD meter.

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Fig. 1. Cyanobacteria sample (A) and cyanobacteria culture (B)

Fig. 2. Experimental setup of hydrophytes-cyanobacteria

2.6.5 Chemical Oxygen Demand (COD)

COD of the filtered sample is measured using the reflux digestion method. 10.0 mL of sample (TWW: Distilled $H_2O = 1:9$) was prepared for each of the TWW samples collected. For each reaction 10.0 mL of the prepared sample was mixed with 5.0 mL of $K_2Cr_2O_7$ sol. (0.25 N), 15.0 mL of AgSO4-H2SO⁴ sol. (10.0 mg/mL), and 0.02 g HgSO⁴ in a digesting tube. Samples were refluxed for 2h and cooled, then the volume was made up to 70.0 mL using distilled H_2O . 8 drops of Ferroin indicator were added to the mixture and titrated against standard FeSO4.(NH4)2SO4.6H2O sol. (0.25 N) until the blue-green colour changes to red wine. The procedure was replicated for the blank.

2.7 Heavy Metal conc. Analysis of TWW Samples

To determine the heavy metals Pb, Cr, Zn, Cu, and Cd, 200 mL of water sample from each setup was gently evaporated until dried. 5.0 mL

of conc. HNO3 was used to dissolve the residues with the subsequent addition of 5–10 drops of H2O² to finish the digestion. 1.0 mL of this solution was used to determine the concentration of heavy metals present using an atomic absorption spectrophotometer (GBC Avanta E, Victoria, Australia; Ser. No. AA7000). Heavy metal conc. analysis of the samples was carried out at the $0th$, 10th and 25th day of the experiment.

3. RESULTS AND DISCUSSION

3.1 Characteristics of TWW Samples

The primary factors that define the properties of tannery effluent are TDS, DO, COD, and Chromate. etc. (Gower, 1980). The permissible limits for collected tannery wastewater samples and their characteristics are explained in Tables 2 and 3. The sample had a TDS of 5150 mg/L and an EC of 10340 µS/cm among the tested parameters. The effluents' pH of 7.9 suggests that they have a slightly alkaline character. The amount of 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 [23, 24]. It was discovered that the TWW samples' TDS, EC, and COD levels, including Cr conc. were above the threshold by an alarming amount. Even the allowed limit for the concentration of other heavy metals, such as 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 3). Therefore, Cd detection was omitted in further analysis.

3.2 Physiochemical Characters of TWW Samples

The collected TWW sample was treated with *Salvinia* (T_{sc}), *Lemna* (T_{LM}), and *Spirulina* (T_{SP}) alone or in combination (T_c) and analyzed for pH, TDS, EC, DO, and COD. These parameters have shown significant changes after various interventions of TWW. Improvement of the effluent after different treatments has been summarized in Table 4. As per the observation, T_{LM} and T_{SP} combination can be recommended for the improvement of the mentioned physiochemical parameters of the tannery effluent.

Table 2. Comparison of physiochemical characters of TWW samples with WHO standards

Parameters	Units	Effluent	Acceptable Limit
pH		7.90	5.50-9.00
TDS	(mg/L)	5150	2100
EC	$(\mu S/cm)$	10340	1200
DO	(mg/L)	2.70	4.50
COD	(mg/L)	8840	250

Table 4. Analysis of the physiochemical characters of the TWW samples throughout the experimentation

3.2.1 Effect of the treatment on the pH level

The TWW's pH was discovered to be 7.9 indicating that the nature of the TWW is slightly alkaline and under the permissible limit of WHO [25]. It is due to the presence of various minerals, including heavy metals. A decrease in the pH is a good indication of the removal of these metals from the effluent. The effluent's pH levels were determined to be 6.42, 6.49, and 6.51 after treatments T_{SC} , T_{LM} , and T_{SP} respectively(Fig. 3). The effluent's pH levels were determined to be 6.93 after the combined treatment (T_c) . Separate treatments resulted in a decrease to its highest level indicating individual treatment of T_{SC} , T_{LM} , and T_{SP} was more effective than combined T_{C} treatment for pH improvement.

3.2.2 Effect of the treatment on the TDS level

The TDS level (5150 mg/L) was found to exceed the permissible limit in the original sample. After T_{SC} , T_{LM} , and T_{SP} treatments, the TDS levels in the effluent were determined to be 3860, 3900, and 3230 mg/L respectively (Fig. 4). The TWW effluent had TDS levels of 4290 mg/L after Tc treatment. The T_{SP} treatment had a significant effect on the maximum decrease in TDS levels, including a steep decrease within the first 10 days of treatment indicating *S. platensis'* potential for rapid TDS reduction.

Fig. 3. Trend of changes in pH levels after treatments of TWW samples

Fig. 4. Trend of changes in TDS levels after treatments of TWW samples

3.2.3 Effect of the treatment on the EC level

The EC level was found 10340 µS/cm in the collected TWW sample exceeding the permissible limit. The effluent had EC levels of 5,600, 4,800, and 4,660 μ S/cm after Tsc, TLM, and T_{SP} treatments respectively (Fig. 5). After T_C treatment, effluent EC levels were 3290 µS/cm, which had the best observed impact for decreasing effluent EC level.

3.2.4 Effect of the treatment on the DO level

Dissolved oxygen (DO) is a very important parameter in water analysis. It is an indicator of the physical, chemical, and biological activities of water. More than 4 mg/L is desirable [26] but the sample showed the amount of DO (2.70 mg/L) below the standard limit in the original sample. The effluent had DO levels of 3.50, 3.63, and 3.75 mg/L after Tsc, TLM, and Tsp treatments respectively (Fig. 6). The T_c treatment however, resulted in DO levels in the effluent being 4.43 mg/L on the 25 th days indicating best-observed impact on the maximum increase in the DO level, which is similar to the prior findings [27].

3.2.5 Effect of the treatment on the COD level

According to WHO prescribed limit for COD is 250 mg/L [25]. The COD level of the TWW sample was found 8840mg/L, which is much higher than the permissible level. The effluent showed COD levels of 1600, 800, and 5000 mg/L after Tsc, TLM, and Tsp treatments respectively (Fig. 7). The effluent showed 1200 mg/L COD levels due to the T_c treatment. However, the maximum drop in COD level was influenced by T_{LM} treatment, including a steep decrease within 10 days of treatment.

Fig. 5. Trend of changes in EClevels after treatments of TWW samples

Fig. 6. Trend of changes in DOlevels after treatmentsof TWW samples

Fig. 7. Trend of changes in COD levels after treatments of TWW samples

3.3 Heavy metal conc. Profile of TWW Samples

Heavy metals were analyzed in both treated and untreated effluent by acid-digesting all the samples. The estimation of heavy metals is presented in Table 5.

3.3.1 Impact of the treatment on the Pb conc.

Pb level was found above the standard limit (0.1718 mg/L) in the collected TWW sample. Following the T_{SC} , T_{LM} , and T_{SP} treatments, the effluent's Pb levels were determined to be 0.124, 0.004, and 0.091 mg/L respectively as shown in Fig. 8. Pb levels in the effluent after the T_c

treatment was found to be 0.082 mg/L. The observation indicates the high efficiency of *L. minor* in scavenging suspending Pb particles in tannery effluent.

3.3.2 Impact of the treatment on the Cr conc.

In the TWW sample, the Cr level (3.438 mg/L) was not within the standard limit. Following the T_{SC}, T_{LM}, and T_{SP} treatments, the levels of Cr were determined to be 1.936, 2.544, and 3.602 mg/L, respectively. Following the Tc treatment, it was found to be 3.005 mg/L(Fig. 9). The observation suggested the high efficiency of *S. cucullata* and *L. minor* in scavenging suspended Cr.

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Fig. 8. Differences in the Pb conc. of TWW samples before and after treatments

Fig. 9. Differences in the Cr conc. of TWW samples before and after treatments

Fig. 10. Differences in the Cu conc. of TWW samples before and after treatments

Fig. 11. Differences in the Zn conc.of TWW samples before and after treatments

3.3.3 Impact of the treatment on the Cu conc.

The Cu level (0.331 mg/L) was above the standard limit in the TWW sample. After T_{SC} , T_{LM} , and T_{SP} treatments, Cu levels of the effluent were found to be 0.183, 0.146, 0.127 mg/L respectively and 0.133 mg/L, after the T_c treatment (Fig. 10). The T_{SP} showed comparatively better Cu level reduction than other treatments.

3.3.4 Impact of the treatment on the Zn conc.

Zn was also found in excess of what was permissible in the TWW sample. Zn concentrations after T_{SC} , T_{LM} , and T_{SP} treatments were found to be 0.032, 0.97, and 0.048 mg/L, respectively. Zn concentrations in the effluent were determined to be 0.084 mg/L after the T_c treatment (Fig. 11). *S. cucullata* and *S. platensis* showed high efficiency in suspended Zn absorption from the tannery effluent.

4. CONCLUSION

Biological remediation of tannery effluent is a cost-effective, environment-friendly, 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 and increase DO. In contrast, the sequences of heavy metals removal were found as follows: by *S.cucullata* Zn>Cr >Pb> Cu, by *L.*

*minor*Pb>Cr > Cu > Zn, by *S. platensis* Cu> Zn>Pb> Cr, and by the combination treatment was $Pb > Cu > Zn > Cr$. Based on the current research, it can be concluded that after the treatments with selected hydrophytes and cyanobacteria alone or in combination, most of the physiochemical parameters decreased below the permissible limit. The observation suggested an overall improvement of tannery effluent quality can be improved by using only the combination of *L. minor and S. platensis.* Further investigation is needed to investigation is needed to the optimal conditions for the bioremediation of Cr and Pb from contaminated sites. Therefore, we are further investigating their combined potential in larger-scale tannery effluent treatment more precisely. Additionally, the observation also implied to investigation of their potential in Arsenic (As) removal as well as their potential of nano-particle production post-treatment of effluent.

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COMPETING INTERESTS

The authors declare that they have no known competing interests (or) personal relationships that could have appeared to influence the work reported in this manuscript.

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