

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

ABSTRACT

Aims: This study aimed to identify the bioremedial 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 interval 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 μ S/cm was observed. Heavy metals removal capacity of the tested species were 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 nano-particle 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,

19 and global warming will be even greater. By 2050, there will likely be a 20.0 to 30.0 % increase in water
20 needed globally for cities, industry, and agriculture [2]. Since the industrial revolution, water pollution has
21 become a growing concern for both the public and societal authorities, as well as one of the most
22 alarming problems the world is currently facing is water contamination with growing industrialization, the
23 issue of heavy metal pollution for wastewater discharge [3]. Even at low concentrations, heavy metals
24 and their related compounds pose major health risks because they are extremely toxic, carcinogenic,
25 mutagenic, and teratogenic [4-7]. Even in small concentrations, heavy metals and their related
26 compounds pose a significant risk to human health because they are extremely toxic, carcinogenic,
27 mutagenic, and teratogenic [8-11]. Hence, Reducing or eliminating heavy metal contamination is crucial
28 for preventing environmental pollution and reducing their uptake and accumulation through the food
29 chain. It is reported that the maximum contaminant levels for Pb, Cr, Cd, Hg, and As in water are 0.01,
30 0.015, 0.1, 0.002, and 0.005, respectively, according to the US Environmental Protection Agency [12,13].
31 Therefore, environmental life is now seriously at risk from heavy metal pollution because of a large
32 amount of heavy metal contamination in water bodies [14]. As a result, numerous health problems such
33 as lung insufficiency, neurological disorders, bone damage, cancer, teratogenic and embryotoxic effects,
34 and hypertension occur in the human body [14,15] According to research conducted thus far, hydrophytes
35 and microalgae have the potential to be a cost-effective and efficient bio-sorbent for the remediation of
36 heavy metal significantly containing wastewater [16,17].

37 Over the past few decades, industry and industrial production growth have expanded rapidly in
38 Bangladesh. Although Bangladesh's industries contribute significantly to the country's economy, they also
39 have a negative impact on the environment. In contrast, the leather and textile industry produced and
40 disposed of crores of litres of untreated wastewater per year. The tannery industry belongs to one of the
41 most polluting industrial sectors. Almost every tannery industry uses significant amounts of chemicals in
42 the process of transforming animal hides into leather [18]. The tanning process is almost completely a wet
43 process that consumes massive amount of water and generates about 90% of the used water as effluent
44 [19]. Tannery effluent carries heavy pollution loads due to a massive presence of highly coloured
45 compounds, sodium chloride and sulphate, various organic and inorganic substances, toxic metallic
46 compounds, different types of tanning materials which are biologically oxidizable and large quantities of
47 putrefying suspended matter. Worldwide, it is estimated that discharged tannery effluent contains 300-
48 400 million tons of heavy metals, solvents, toxic sludge and other wastes, which are dumped into the
49 water each year [19]. Treatment of tannery wastewater is carried out by physical, chemical, biological, or
50 combinations of these methods. A principal factor of water pollution contributes to oxygen demand and
51 nutrient loading in the water bodies, promoting algal biomass and damaging the aquatic ecosystem [18].

52 Bioremediation is one of the important pollution control technologies that use some biological systems to
53 degrade various toxic chemicals into less harmful forms. Besides this, bioremediation may be defined in
54 many other ways. According to the Environmental Protection Act (EPA 1976) [20], bioremediation is a
55 "Treatment that uses naturally occurring organisms and plants to breakdown dangerous substances into
56 non-toxic substances". Cyanobacteria is found to be an important biological agent for wastewater
57 treatment. *Spirulina* sp. is a cyanobacterium that grows rapidly contains a detectable level of Mercury and
58 Lead when grown under contaminated conditions, implying that it can take up toxic metals from the
59 environment [21]. The hydrophytes *Lemna* sp., *Salvinia* sp., *Pistia* stratiotes and *Eichhornia crassipes* are
60 a major part of the aquatic ecosystem and show high removal efficiency for Hg, Pd, Zn, Cu, and Mn. They
61 adsorb the contaminants and translocate their roots and shoots from polluted wastewater [22] and other
62 several aspects [28-37].

63 This study is focused on assessing the quality level of the tannery effluent that is regularly discharged into
64 municipal drains and ultimately ends up in rivers. However, to solve this problem, a variety of methods
65 can be used, but they are expensive and harmful to the environment. Therefore, cyanobacteria and
66 hydrophytes may be cost-efficient and useful bio-sorbent for cleaning up heavy metal wastewater. It
67 produces valuable biological materials for the synthesis of bioactive compounds and biofuels and
68 recycles nutrients efficiently. This manuscript explores the use of cyanobacteria and hydrophytes for

69 heavy metal bioremediation, discusses strategies for improvement, and presents potential challenges for
70 a greener environment.

71 2. METHODOLOGY

72 2.1. Collection of tannery effluent

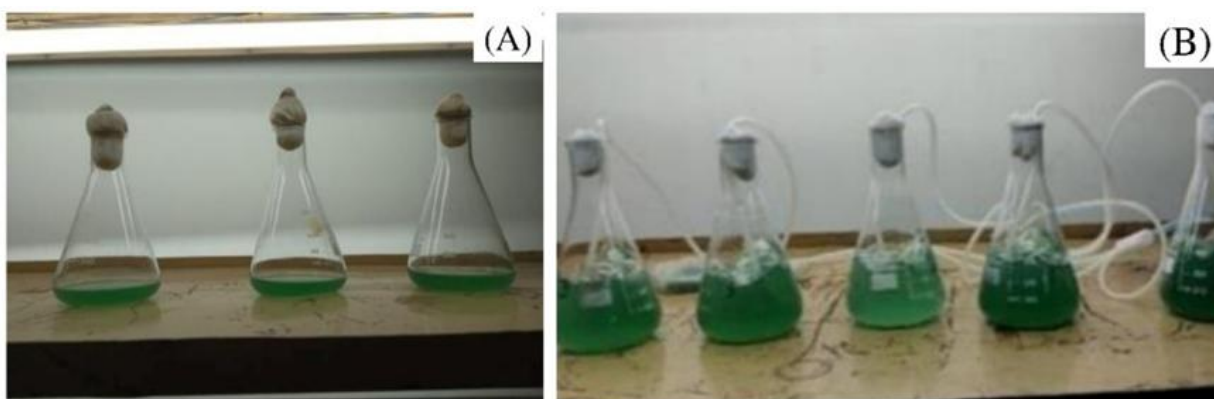
73 The tannery wastewater (TWW) was collected from Ruma Leather Industries Ltd., located at Savar,
74 Dhaka, Bangladesh. Samples were collected four times throughout the study period from the industry's
75 treatment plant outlet pipes. During collection, wastewater samples were found to be coloured along with
76 an obnoxious and unpleasant odour. 5L plastic air-tight containers were used to collect the samples
77 and preserved at room temperature until used.

78 2.2. Collection of hydrophytes

79 We selected two types of locally available free-floating hydrophytes *Salvinia cucullata* Roxb. (Family-
80 Salviniaceae) and *Lemna minor* (Family- Araceae) for the treatment of TWW. *Salvinia cucullata* and
81 *Lemna minor* were collected from a freshwater pond of Kashiani, Gopalganj and characterized in the
82 Applied Botany Laboratory, Dhaka Laboratory, BCSIR. The hydrophytes were preserved in tap water at
83 room temperature without any nutrients for two weeks in earthen containers with a depth of 0.3m and a
84 capacity of 40L each.

85 2.3. Collection of microalgae

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



88
89 Fig. 1. Cyanobacteria sample (A) and cyanobacteria culture (B)

90 2.4. Culture of microalgae

91 *Spirulina platensis* was cultured in Zarrouk's medium of pH 9.5 at $(25 \pm 1)^\circ\text{C}$ following aseptic conditions.
92 The culture was gently mixed and exposed to white light, which was produced at a rate of 50.0mmol
93 $\text{photon}/\text{m}^2/\text{s}$ using cool white fluorescent tubes (Fig. 1(B)). The light/dark cycle of 14:10 hours was
94 maintained. The reagents for Zarrouk's medium (as mentioned in Table 1) were provided by Applied
95 Botany Laboratory, Dhaka Laboratory, BCSIR.

96 Table 1. Composition of Zarrouk's medium

Chemicals	Amount (g/L)
NaCl	1.00
NaNO ₃	2.50
K ₂ SO ₄	1.00
NaHCO ₃	16.8
K ₂ HPO ₄	0.50

MgSO 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 (H ₃ BO ₃ , MnCl ₂ ·4H ₂ O, ZnSO ₄ ·4H ₂ O, Na ₂ MoO ₄ , CuSO ₄ ·5H ₂ O)	1.00 mL

97

98 **2.5. Experimental Design for TWW Treatment**

99 The wastewater was filtered through the Whatman filter paper No.4. and samples (TWW:distilled H₂O =
100 7:3) were prepared. The experiment included three different individual treatments of the prepared TWW
101 samples (T_{SC}, T_{LM}, T_{SP}), one combined treatment (T_C) and one control (C). Each setup contained 1L
102 sample and each treatment was performed in triplicates for 25 days.



103

104 **Fig. 2. Experimental setup of hydrophytes-cyanobacteria**

105 **2.5.1. Treatment with hydrophytes**

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

109 **2.5.2. Treatment with microalgae**

110 *S.platensis* was used separately in another set of experiments (T_{SP}) with 20.0 mL of wet *S.platensis* per litre
111 of water sample for treatment.

112 **2.5.3. Combined treatment with hydrophytes and microalgae**

113 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
114 mL of wet *S.platensis*, per liter of water sample for treatment.

115 **2.6. Physicochemical analysis of TWW samples**

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

121 **2.6.1. pH**

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

125 **2.6.2. Total Dissolved Solid (TDS)**

126 The filtered sample was used for measuring the TDS using the Hanna Instruments: HI 9829 multi-
127 parameter.

128 **2.6.3. Electrical Conductivity (EC)**

129 The filtered sample was used for measuring the EC using the Hanna Instruments: HI 9829 multi-
130 parameter.

131 **2.6.4. Dissolved Oxygen (DO)**

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

133 **2.6.5. Chemical Oxygen Demand (COD)**

134 COD of the filtered sample is measured using the reflux digestion method. 10.0 mL of sample (TWW:
135 Distilled H₂O = 1:9) was prepared for each of the TWW samples collected. For each reaction 10.0 mL of
136 the prepared sample was mixed with 5.0 mL of K₂Cr₂O₇ sol. (0.25 N), 15.0 mL of AgSO₄-H₂SO₄ sol. (10.0
137 mg/mL), and 0.02 g HgSO₄ in a digesting tube. Samples were refluxed for 2h and cooled, then the volume
138 was made up to 70.0 mL using distilled H₂O. 8 drops of Ferroin indicator were added to the mixture
139 and titrated against standard FeSO₄·(NH₄)₂SO₄·6H₂O sol. (0.25 N) until the blue-green color changed to red
140 wine. The procedure was replicated for the blank.

141 **2.7. Heavy metal conc. analysis of TWW samples**

142 To determine the heavy metals Pb, Cr, Zn, Cu, and Cd, 200 mL of water sample from each setup was
143 gently evaporated until dried. 5.0 mL of conc. HNO₃ was used to dissolve the residues with the
144 subsequent addition of 5–10 drops of H₂O₂ to finish the digestion. 1.0 mL of this solution was used to
145 determine the concentration of heavy metals present using an atomic absorption spectrophotometer
146 (GBC Avanta E, Victoria, Australia; Ser. No. AA7000). Heavy metal conc. analysis of the samples was
147 carried out at the 0th, 10th and 25th day of the experiment.

148 **3. RESULTS AND DISCUSSION**

149 **3.1. Characteristics of TWW samples**

150 The primary factors that define the properties of tannery effluent are TDS, DO, COD, and Chromate. etc.
151 (Gower, 1980). The permissible limits for collected tannery wastewater samples and their characteristics
152 are explained in **Tables 2 and 3**. The sample had a TDS of 5150 mg/L and an EC of 10340 µS/cm among
153 the tested parameters. The effluents' pH of 7.9 suggests that they have a slightly alkaline character. The
154 amount of DO was 2.70 mg/L. It was found that the chemical oxygen demand was 8840 mg/L. Heavy
155 metals, including Cr, Cu, Pb, and Zn were found in the tannery effluent, with respective values of 8.43,
156 0.33, 0.17, and 1.32 mg/L. Other researchers have reported the majority of these findings [23, 24]. It was
157 discovered that the TWW samples' TDS, EC, and COD levels, including Cr conc. were above the
158 threshold by an alarming amount. Even the allowed limit for the concentration of other heavy metals, such
159 as Pb, Cu, and Zn, was exceeded. Nonetheless, the TWW sample's Cd content was found to be within
160 the permitted limit (0.006 mg/L) (**Table 3**). Therefore, Cd detection was omitted in further analysis.

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

Parameters	Units	Effluent	Acceptable Limit
pH		7.9	5.5-9.0
TDS	(mg/L)	5150	2100
EC	(µS/cm)	10340	1200
DO	(mg/L)	2.70	4.50
COD	(mg/L)	8840	250

162 **Table 3. Comparison of heavy metal conc. profile of TWW samples with WHO standards**

Parameters	Units	Effluent	Acceptable Limit
Pb	(mg/L)	0.1718	0.10

Cr	(mg/L)	8.4380	2.00
Cd	(mg/L)	0.0064	2.00
Cu	(mg/L)	0.3312	0.10
Zn	(mg/L)	1.3241	1.00

163

164 **3.2. Physiochemical characters of TWW samples**

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

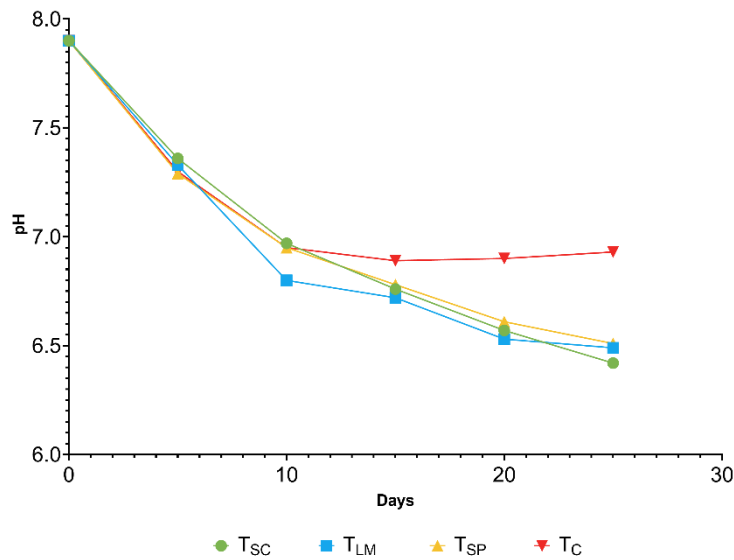
170 **Table 4. Analysis of the physiochemical characters of the TWW sample throughout the**
 171 **experimentation**

Treatment	Parameter	Unit	Days					
			0	5	10	15	20	25
T_{SC}	pH		7.9	7.36	6.97	6.76	6.57	6.42
	TDS	(mg/L)	5150	4800	4530	4300	3900	3860
	EC	(μ S/cm)	10340	8900	6800	4660	5000	5600
	DO	(mg/L)	2.70	2.81	2.89	2.96	3.07	3.50
	COD	(mg/L)	8840	6220	4900	3600	2220	1600
T_{LM}	pH		7.9	7.33	6.80	6.72	6.53	6.49
	TDS	(mg/L)	5150	4900	4440	4330	4200	3900
	EC	(μ S/cm)	10340	7800	6000	5600	5300	4800
	DO	(mg/L)	2.70	2.95	3.07	3.21	3.36	3.63
	COD	(mg/L)	8840	4600	2000	1200	1000	800
T_{SP}	pH		7.9	7.29	6.95	6.78	6.61	6.51
	TDS	(mg/L)	5150	4500	3340	3660	3560	3230
	EC	(μ S/cm)	10340	5420	4980	3800	4300	4660
	DO	(mg/L)	2.70	2.83	3.08	3.36	3.63	3.75
	COD	(mg/L)	8840	7600	5000	6600	7000	6660
T_C	pH		7.9	7.30	6.95	6.89	6.90	6.93
	TDS	(mg/L)	5150	4900	4760	4660	4330	4290
	EC	(μ S/cm)	10340	5960	4400	4560	3341	3290
	DO	(mg/L)	2.70	3.26	3.53	3.75	3.96	4.43
	COD	(mg/L)	8840	5000	3800	2500	1600	1200

172

173 **3.2.1. Effect of the treatment on the pH level**

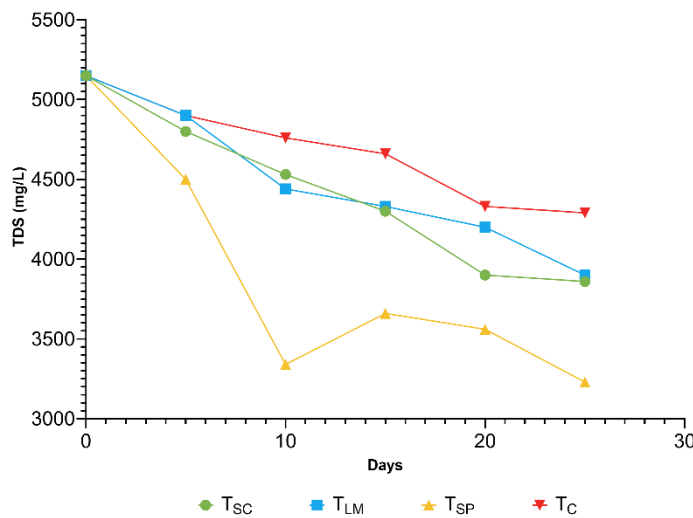
174 The TWW's pH was discovered to be 7.9 indicating that the nature of the TWW is slightly alkaline and
 175 under the permissible limit of WHO [25]. It is due to the presence of various minerals, including heavy
 176 metals. A decrease in the pH is a good indication of the removal of these metals from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



181
182 **Fig. 3. Trend of changes in pH levels after treatments of TWW samples**

183 **3.2.2. Effect of the treatment on the TDS level**

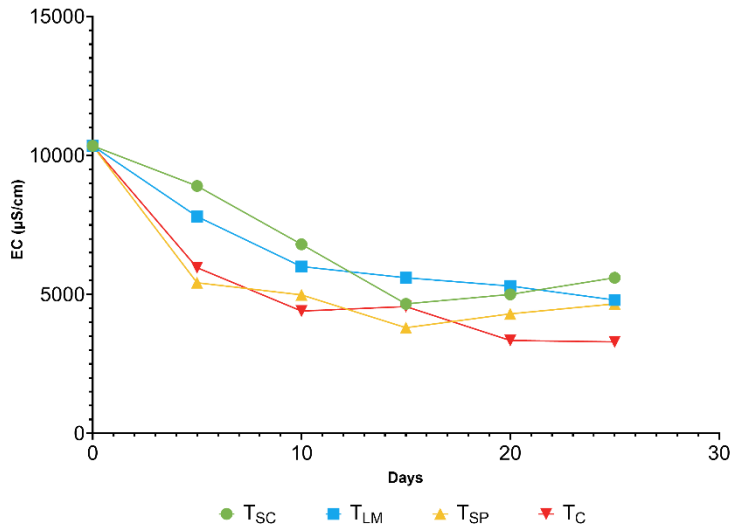
184 The TDS level (5150 mg/L) was found to exceed the permissible limit in the original sample. After T_{SC},
185 T_{LM}, and T_{SP} treatments, the TDS levels in the effluent were determined to be 3860, 3900, and 3230 mg/L
186 respectively (Fig. 4). The TWW effluent had TDS levels of 4290 mg/L after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



189
190 **Fig. 4. Trend of changes in TDS levels after treatments of TWW samples**

191 **3.2.3. Effect of the treatment on the EC level**

192 The EC level was found 10340 $\mu\text{S}/\text{cm}$ in the collected TWW sample exceeding the permissible limit. The
193 effluent had EC levels of 5,600, 4,800, and 4,660 $\mu\text{S}/\text{cm}$ after T_{SC}, T_{LM}, and T_{SP} treatments respectively
194 (Fig. 5). After T_C treatment, effluent EC levels were 3290 $\mu\text{S}/\text{cm}$, which had the best-observed impact for
195 decreasing effluent EC level.

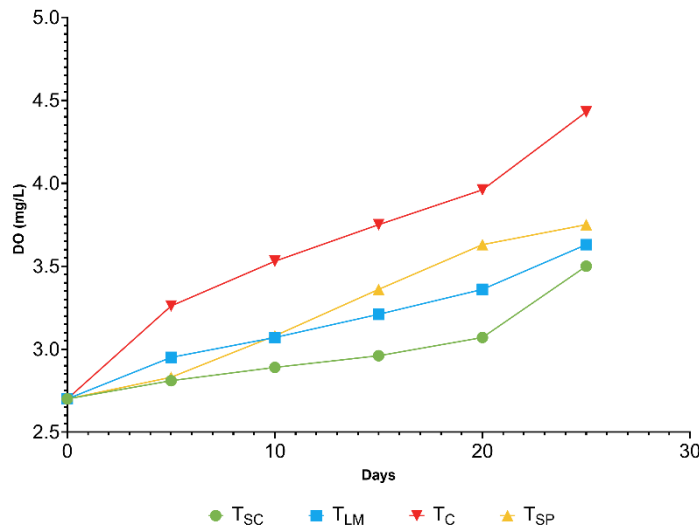


196

197 **Fig. 5. Trend of changes in EC levels after treatments of TWW samples**

198 **3.2.4. Effect of the treatment on the DO level**

199 Dissolved oxygen (DO) is a very important parameter in water analysis. It is an indicator of the physical,
 200 chemical, and biological activities of water. More than 4 mg/L is desirable [26] but the sample showed the
 201 amount of DO (2.70 mg/L) below the standard limit in the original sample. The effluent had DO levels of
 202 3.50, 3.63, and 3.75 mg/L after T_{SC}, T_{LM}, and T_{SP} treatments respectively (Fig. 6). The
 203 T_C treatment however, resulted in DO levels in the effluent being 4.43 mg/L on the 25th day indicating best-
 204 observed impact on the maximum increase in the DO level, which is similar to the prior findings [27].

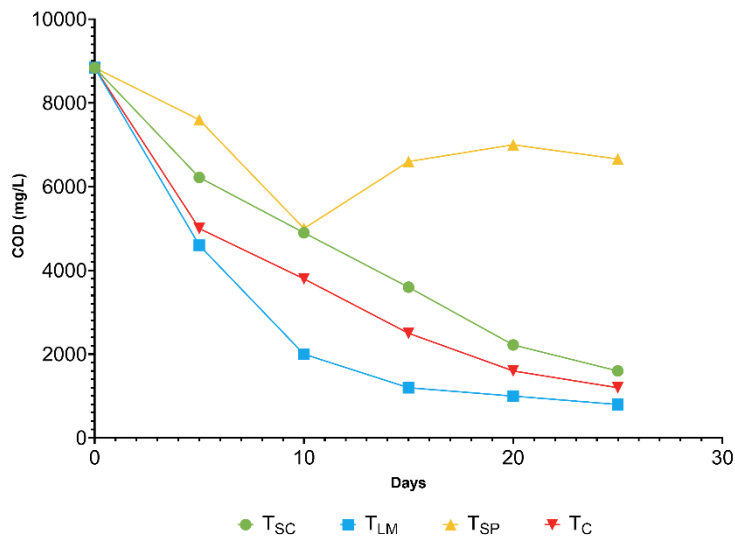


205

206 **Fig. 6. Trend of changes in DO levels after treatments of TWW samples**

207 **3.2.5. Effect of the treatment on the COD level**

208 According to WHO prescribed limit for COD is 250 mg/L [25]. The COD level of the TWW sample was
 209 found 8840 mg/L, which is much higher than the permissible level. The effluent showed COD levels of
 210 1600, 800, and 5000 mg/L after T_{SC}, T_{LM}, and T_{SP} treatments respectively (Fig. 7). The effluent showed
 211 1200 mg/L COD levels due to the T_C treatment. However, the maximum drop in COD level was influenced
 212 by T_{LM} treatment, including a steep decrease within 10 days of treatment.



213

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

215 **3.3. Heavy metal conc. profile of TWW samples**

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

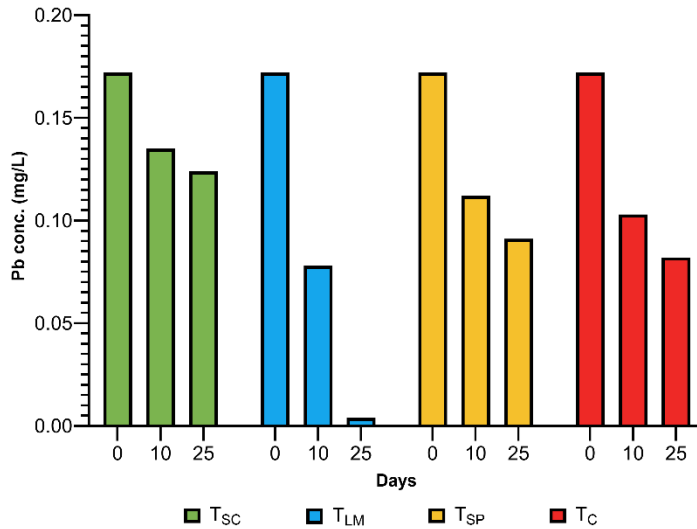
218 **Table 5. Analysis of the heavy metal conc. of the TWW samples throughout the**
 219 **experimentation**

Treatments	Heavy metals	Unit	Days		
			0	10	25
T _{SC}	Pb	(mg/L)	0.172	0.135	0.124
	Cr		8.438	6.523	1.936
	Cu		0.331	0.246	0.183
	Zn		1.324	1.003	0.032
T _{LM}	Pb		0.172	0.078	0.004
	Cr		8.438	7.637	2.544
	Cu		0.331	0.215	0.146
	Zn		1.324	1.278	0.97
T _{SP}	Pb		0.172	0.112	0.091
	Cr		8.438	7.962	3.602
	Cu		0.331	0.194	0.127
	Zn		1.324	1.045	0.048
T _C	Pb		0.172	0.103	0.082
	Cr		8.438	7.841	3.005
	Cu		0.331	0.203	0.133
	Zn		1.324	1.109	0.084

220

221 **3.3.1. Impact of the treatment on the Pb conc.**

222 Pb level was found above the standard limit (0.1718 mg/L) in the collected TWW sample. Following the
 223 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
 224 respectively as shown in **Fig. 8.** Pb levels in the effluent after the T_C treatment were found to be 0.082
 225 mg/L. **The observation indicates the high efficiency of *L. minor* in scavenging suspending Pb particles in**
 226 **tannery effluent.**

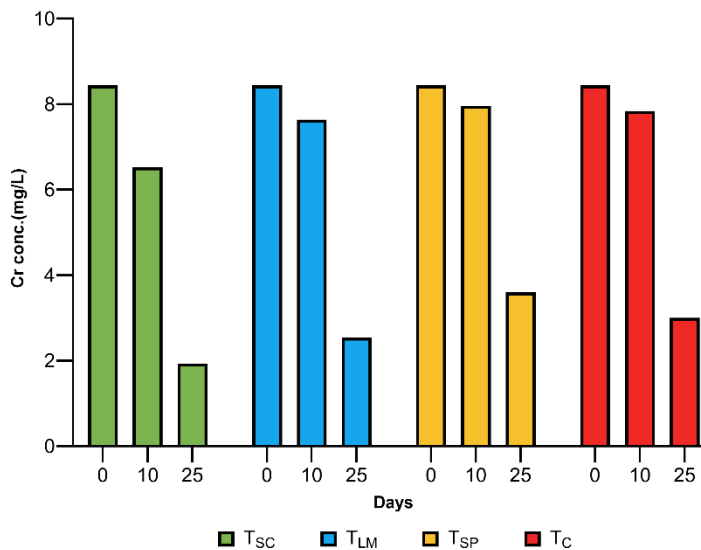


227

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

229 **3.3.2. Impact of the treatment on the Cr conc.**

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

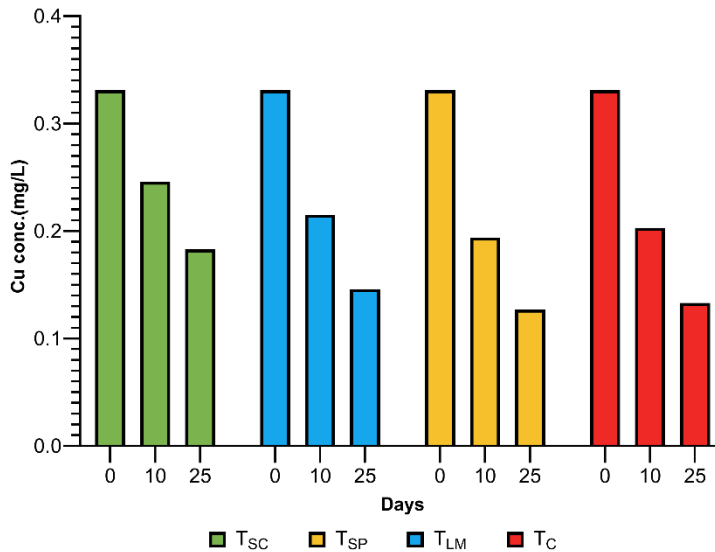


234

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

236 **3.3.3. Impact of the treatment on the Cu conc.**

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

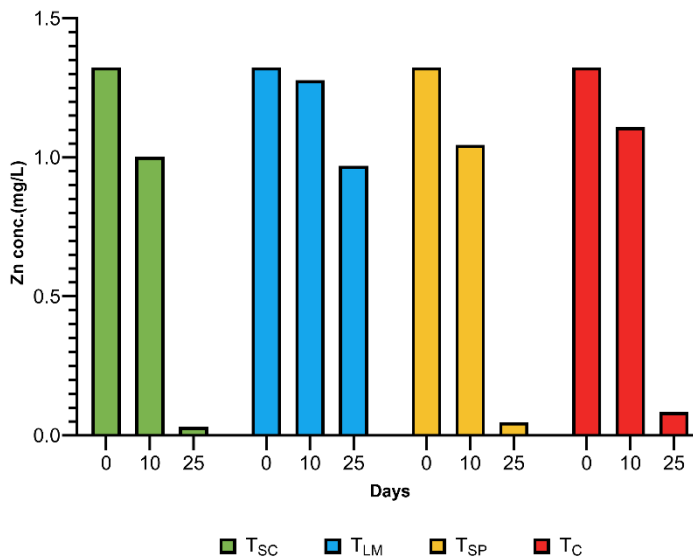


241

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

243 **3.3.4. Impact of the treatment on the Zn conc.**

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



248

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

250 **4. CONCLUSION**

251 Biological remediation of tannery effluent is a cost-effective, environment-friendly, and easily accessible
 252 method that significantly removes heavy metals from the environment. The study suggests that treating
 253 industrial wastewater with hydrophytes (*Salvinia cucullata* and *Lemna minor*) and cyanobacteria (*Spirulina*
 254 *platensis*) can effectively remove pollutants, reduce COD, TDS, and EC and increase DO. In contrast, the
 255 sequences of heavy metals removal were found as follows: by *S. cucullata* Zn > Cr > Pb > Cu, by

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

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272 COMPETING INTERESTS

273 The authors declare that they have no known competing interests (or) personal relationships that could
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275 AUTHORS' CONTRIBUTIONS

276 NNH: Conceptualization, Methodology, Supervision, Writing- Original Draft, Writing- Review & Editing;
277 MAA: Investigation, Software, ASB: Data curation, Visualization; MEAZ: Methodology, Resources; MZR:
278 Visualization; CKR: Review; and JLM: Supervision, Project Administration, Funding Acquisition.

279 DATA AVAILABILITY

280 All data created for this research are provided within the article/supplementary material; further enquiries
281 can be directed to the corresponding author(s).

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