Heavy Metal Scavenging Potential of Indigenous Microalgae of Bangladesh: A Study on itsApplication in Textile Effluent Treatment

ABSTRACT

Aims: The aim of study was to identify the physicochemical property improvement and heavy metal scavenging potential of indigenous microalgae(*Spirulina sp.* and *Chlorella sp.*) of Bangladesh for treatment of textile wastewater disposed in the open environment.

Study design: The capacity of improving the water quality of the textile effluent by heavy metal absorption was assessed. The quantitative determination included the comparison of physical characters (pH, TDS, EC, DO, COD) and heavy metal profile (Cr, Cd, Pb, Zn, Fe) of the textile effluent before and after bioremediation. Effluent treatment was carried out by individual species separately and in combination of both 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 textile wastewater was characterized by means of physicochemical parameters and heavy metal concentration prior to the experimental procedure. Three treatment plans were designed, two (T_{CV} and T_{SP}) using individual species separately and one treatment (T_C) using both the species in combination. The treatment continued for 25 days. The physicochemical parameters and heavy metal concentration of the treated effluent were measured at 5-day interval till the 25^{th} day of the experiment. Comparative analysis of the data was utilized to determine useful species for further applied studies in future.

Results: The *Chlorella* treatment achieved remarkable pH restoration, with a peak of 7.94 on Day 10 and stabilization at 7.28 by Day 25. Moreover, the same treatment exhibited substantial reductions in TDS, dropping from 7750 mg/L to 455 mg/L by Day 10, and further to 301 mg/L by Day 25. Additionally, it effectively reduced heavy metal concentrations; Cr from 0.783 ppm to 0.462 ppm by Day 25, well below permissible limits.

Conclusion: The comparative data suggested the overall improvement of the effluent quality can be achieved by a combined treatment of *C. vulgaris* and *S. platensis*, quickly and cost-efficiently. Further investigation is required for an in-depth understanding of their combined potential.

Keywords: *Textile effluent, Microalgae, Spirulina, Chlorella, Heavy metal scavenging, Wastewater treatment*

1. INTRODUCTION

The increasing global population has presented numerous challenges to the world economy, particularly regarding environmental preservation and energy security. Discharged textile wastewater pollutes rivers, lakes and seas worldwide [1]. There is growing concern about the substantial volume of effluents released from textile processing which consumes large amounts of water [2]. Untreated effluent from nearby textile factories has been discharged into rivers with major contaminants located outside the area such as in Narayangonj, Savar and Chattogram in some industrial areas. The annual global production of dyestuff exceeds 700,000 tonnes [3].The textile industry uses large amounts of water for various stages of dyeing and cleaning raw materials [4]. As a result, the wastewater from textile production contains harmful heavy metals such as cadmium (Cd), chromium (Cr) and lead (Pb). These toxic metals threaten living organisms, including humans, due to their biotoxic effectswhich can be acute, chronic, sub-chronic, neurotoxic, carcinogenic, mutagenic or teratogenic [5,41.42,43]. For example, even low levels of cadmium can be extremely toxic, leading to bone defects, increased blood pressure, myocardial dysfunctions, pulmonary oedema and, in severe cases, death [6]. Studies have shown that lead is the most significant of the toxic heavy metals, being absorbed in inorganic forms through ingestion of food and water as well as inhalation [7]. Lead poisoning can cause inhibition of haemoglobin synthesis, kidney and joint dysfunctions, reproductive system issues, and acute and chronic damage to the central nervous system [8]. Textile wastewater is a major source of surface water contamination and various technologies are being developed for treating these effluents. Among these technologies, adsorption is considered one of the most promising

methods [9, 10]. Recent studies have focused on the adsorptive removal of heavy metals and dyes using chitosan-based materials. [11, 12].

Four common ways to treat wastewater include physical water treatment, biological water treatment, chemical treatment and sludge treatment. Agents used for bioremediation are bacteria, fungiand algae [13]. Microalgae are reported in many studies to alleviate heavy metal toxicity. In recent years, the use of microalgae and cyanobacteria in the bioremediation of coloured wastewater has attracted interest due to their central role in carbon dioxide fixation. In addition, the generated algae biomass has potential as feedstock for biofuel production. Algal ability to remove dyes from wastewater can be enhanced by stimulating their growth. Living biomass of microalgae such as Chlorella sp. can remove 63.0 – 69.0 % of the colour from the mono-azo dye tectilon yellow 2G by converting it to aniline [14]. Microalgae such as Chlorella sp. and Spirulina sp. grown respectively in CH medium and Zarrouk's medium are demonstrated to be useful in treating effluent textile wastewater [15]. Harvesting is the crucial step in the production of algal biomass, as it accounts for 20.0 – 30.0% of production costs. The small size of microalgae (3.0 – 30.0 μm) and its low concentration in the culture medium (below 500.0 mg/L) make cell recovery a very challenging process. Several species of algae with varying characteristics like shape, size and motility influence their settling.

Chlorella is a microscopic green alga, spherical or ellipsoidal not much larger than a red blood cell. The name of this single-celled, non-motile water plant comes from the Greek chloros=green or yellow-green and ella=small. The cells are usually 2.0 – 12.0 μm in diameter but the size can vary, even within a single population. The cells are solitary or in irregular clumps. Chlorella has a high growth rate, making it very interesting for research in various fields [15]. There are various areas where Chlorella is used, such as to remove dyes by bio-adsorption, biodegradation

and bioconversion. Chlorella sp. can degrade dyes by removing nitrogen, phosphorus and carbon from water [16].

Spirulina sp. is another organism whose role as an effective material to scavenge metal ions is exclusively examined [17, 18]. Many studies have aimed to analyze the tolerance and absorption mechanisms of toxic metals such as Cr, Cd and copper (Cu) in Spirulina sp. [19]. It is also characterized by a higher capacity to remove effluents from textile wastewater [20]. Textile effluents are the causes that reduce the nutrients of water bodies. It is well known for creating biofilms on the water's surface so that the lack of sunlight causes aquatic life to suffer [21]. Many studies examined the impact[26-40] of various dyes on water, concluding that higher dye concentrations inhibit the growth of Spirulina sp. and reduce its nutrient levels [22].

Bioremediation has become the primary choice for contaminated site recovery in America. It is commonly used globally for situations where previous human activity has left the location damaged and unusable without remediation [23]. With the country's population rising, the demand for landfills to relocate polluted material surpasses the available supply [24]. On the other hand, biological treatment could achieve greater efficiencies in the decolourization and detoxification of textile wastewater by using native aquatic plants [25]. Using microorganisms to break down pollutants or waste, such as oil spills, contaminated groundwater or industrial processes makes bioremediation a very attractive solution [26].

This study focused on the growth parameter optimization of Spirulinasp. and Chlorella sp. biomass production as one of the key factors influencing heavy metal removal from industrial wastewater. There were few studies of heavy metal scavenging in the past, under a consortium condition of Spirulina sp. and Chlorella sp. We addressed fundamental physicochemical changes

of textile effluent under consortium conditions, including its efficiency in scavenging Cr, Cd, Pb, zinc (Zn), and iron (Fe).

2. MATERIALS &METHODS

2.1. Textile effluent collection

Textile wastewater was collected from the textile mills in Narayangonj, Bangladesh. The liquid is collected successively in four 5.0 L gallons that have retention times of 2.0 to 3.0 days depending on water use, weather and land application practices. The gallons were maintained in anaerobicconditions and mixed vertically. The samples were not stratified concerning pH, temperature or electrical conductivity. The water also had a dark-brown colour (Fig. 1B).

Fig. 1. Textile wastewater samples (A) Collection area (B) Collected wastewater.

2.2. Preservation of the sample

Wastewater preservation techniques were used to prevent retardation of the chemicals and biological changes that usually continues after the samples have been collected. No preservatives were used during the transportation of the samples to the laboratory. Around 20.0 L effluent

sampleswere collected (in fresh plastic gallons). 5.0 mL of conc. HNO_3 was added to each effluent sample bottle to prevent air oxidation and was preserved in a 4.0 ˚C refrigerator.

2.3. Collection of microalgae

The Applied Botany Laboratory, BCSIR, Dhaka Laboratories, from its specialized raceway culture ponds (Fig. 2), provided the cyanobacteria *Spirulina platensis* and *Chlorella vulgaris*.

Fig. 2. *Spirulina platensis*raceway pond at BCSIR Laboratories, Dhaka

2.4. Culture of microalgae

Both *Spirulina platensis*and *Chlorella vulgaris* were cultured in 100 mL culture media in 500 mL Erlenmeyer flasks at 23.0 ± 1.0 ˚C following aseptic conditions. The cultures were gently agitatedover an orbital shaker (SYC-2102) and exposed to white light for a24.0 hours photoperiod by using a cool white fluorescent light. Cell growth was monitored by determination of optical density (OD_{750}) at 750.0 nm. For *Spirulina* culture (Fig. 3A),Zarrouk's medium of pH 9.50(Table 1) was used and for *Chlorella*culture (Fig. 3B), CH medium was used (Table 2). The Applied Botany Laboratory, BCSIR Dhaka Laboratories, provided the reagents for media preparation.

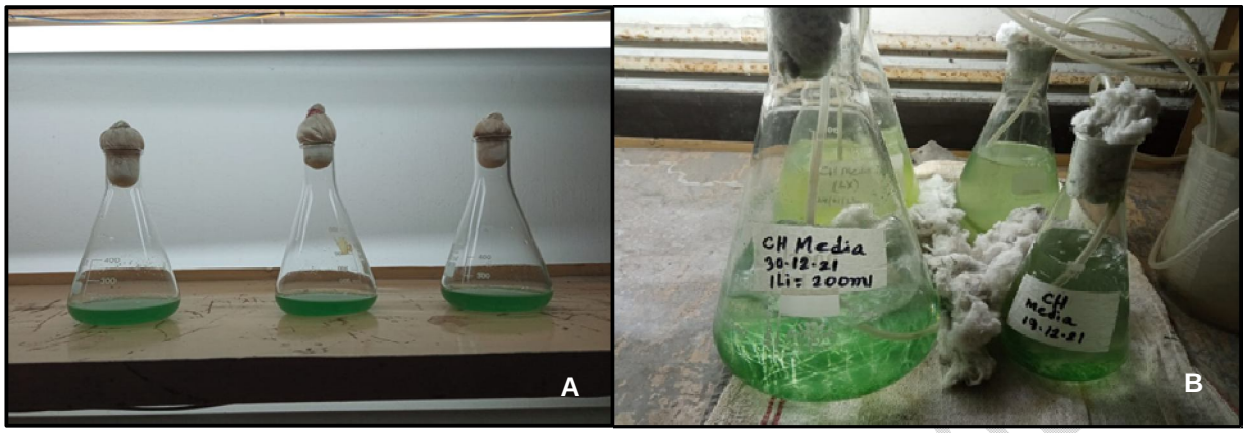

Fig. 3. Flask culture of (A) *Spirulina platensis*in Zarrouk's medium and (B) *Chlorella vulgaris* in CH medium

Table 1. Composition of Zarrouk's medium

Chemicals	Amount (g/L)
NaCl	1.00
$NaNO_3$	2.50
K_2SO_4	1.00
$NaHCO_3$	16.8
K_2HPO_4	0.50
$MgSO_4.7H_2O$	0.20
$FeSO_4.7H_2O$	0.01
$CaCl_2.2H_2O$	0.04
EDTA-$Na_2.2H_2O$	0.08
A_5 Micro Nutrient (H_3BO_3, $MnCl_2.4H_2O$, $ZnSO_4.4H_2O$, Na_2MoO_4, $CuSO_4.5H_2O$)	1.00 mL

Table 2. Composition of CH medium

Chemicals	Amount (g/L)
KNO_3	6.00
K_2HPO_4	0.24
$MgSO_4$	0.06
$FeSO_4$	0.03
$CaSO_4.2H_2O$	0.012

2.5. Experimental design for the effluent treatment

The study used twelve glass beakers (1.0 – 2.0 L) representing three treatments and one control in three replicates. All aquaria were filled with 1.0 L of water from textile wastewater samples. The control treatment consisted solely of textile wastewater. Treatments were set up in a 1.0 – 2.0 L glass beaker in normal daylight and temperature. Each set up of wastewater and microalgae amounts of 1.0 L wastewater with 10.0 mL of pure microalgae like *Spirulina* sp. (T_{SP}), *Chlorella* sp. (T_{CV}), and Combined (T_C). The growth of microalgae in the five treatments was quantified in terms of cell count by using a T80 UV-visible spectrophotometer (OD_{600}, PG Instruments, United Kingdom). No artificial shaker is used in this treatment method. Treatments were carried out for 25 days and data was collected from all samples at 5 days intervals.

2.6. Physicochemical analysis of the samples

For textile wastewater analysis, 100.0 mL samples were taken every five days from each glass aquarium and were placed in plastic bottles. These samples show some different conditions when it was treated with microalgae.

2.6.1. pH

The pH meter is calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.18 at room temperature. When testing water samples, the pH is determined at room temperature. Before each sample measurement, the electrode is thoroughly washed with distilled water and cleaned with tissue paper. Approximately 100.0 mL of the sample is taken in a glass beaker. The electrode is then dipped into the sample. The instrument provides a direct measurement of pH. The pH of the filtered sample is measured using the HachSensION 4-Thermo Fisher.

2.6.2. Salinity

The salinity of water samples is determined at room temperature. The electrode is thoroughly washed with distilled water and cleaned with tissue paper before each measurement of the sample and buffer solutions. Approximately 100.0 mL of the sample is taken in a glass beaker. The electrode is then immersed in the sample. The HachSensION 4-Thermo Fisher provides a direct measurement of salinity.

2.6.3. Total Dissolved Solid (TDS)

The total dissolved solids (TDS) of water samples are determined at room temperature. The electrode is washed thoroughly with distilled water and cleaned with tissue paper before each

measurement of the sample and buffer solutions. About 100.0 mL of the sample is taken in a glass beaker. The electrode is then dipped in the sample, and the instrument provides a direct measurement of TDS. The filtered sample was used for measuring the TDS using the HachSensION 4-Thermo Fisher.

2.6.4. Electrical Conductivity (EC)

The conductivity cell should be washed thoroughly with distilled water and cleaned with tissue paper before each measurement, whether it's for the sample or the KCl standard solution. All conductance measurements should be taken at a temperature of 25.0 ± 0.10 °C. To calibrate, the conductivity cell needs to be placed in the standard KCl solution. When taking a sample measurement, 100.0 mL of the sample should be poured into a 100.0 mL glass beaker. Then, the conductivity cell should be submerged in the beaker and the EC value noted. The EC of the filtered sample should be measured using the HachSensION 4-Thermo Fisher.

2.6.5. Dissolved Oxygen (DO)

The dissolved oxygen (DO) meter is calibrated using standard solutions at room temperature. Before each measurement, the electrode is washed thoroughly with distilled water and cleaned with tissue paper. Approximately 100.0 mL of the sample is taken in a glass beaker and then the electrode is dipped into the sample. The instrument provides a direct measurement of dissolved oxygen. For measuring the DO, the filtered sample was used with the HACH Instrument HQ 30D meter.

2.6.6. Chemical Oxygen Demand (COD)

The COD of the filtered sample is measured using the reflux digestion method. For each of the TWW samples collected, 10.0 mL of sample (Sample:Distilled H_2O = 1:9) was prepared. For each reaction, 10.0 mL of the prepared sample was mixed with 5.0 mL of $K_2Cr_2O_7$ solution (0.25 N), 15.0 mL of $AgSO_4$-H_2SO_4 solution (10.0 mg/mL), and 0.02 g $HgSO_4$ in a digesting tube. The samples were refluxed for 2.0 hours, then cooled, and the volume was made up to 70.0 mL using distilled H_2O. Next, eight (8) drops of Ferroin indicator were added to the mixture and it was titrated against standard $FeSO_4.(NH_4)_2SO_4.6H_2O$ solution (0.25 N) until the blue-green colour changes to red wine. The procedure was also carried out for the blank sample.

2.7. Heavy metal concentration analysis

To analyze the heavy metals Cr, Cd, Pb, Zn, and Fe, 200 mL of water sample from each setup was gently evaporated until dried. The residues were dissolved with 5.0 mL of concentrated HNO_3, and 5.0 –10.0ml of H_2O_2 were added to complete the digestion process. Then, 1.0 mL of this solution was used to determine the concentration of heavy metals using an atomic absorption spectrophotometer (PerkinElmer "AAnalyst 700") and the program operated by the software AAwinlab Analyst-v4.1.

On the instrument side, the air and acetylene were manually started, and the instrument was switched on. After achieving the proper pressure of air and acetylene (75.0 and 30.0 kg/cm^2, respectively), the burner was ignited. Calibration involved setting the atomization position for the required absorbance standard. Standard solutions of 5.0 and 10.0 mg/L of a particular metal were applied and a linear graph appeared. Once the calibration curve was satisfactory, the instrument was ready for sample measurement.

During sample measurement, the sample blank was aspirated, and distilled water was aspirated after every measurement. The concentration in mg/L was recorded directly from the screen.

3. RESULTS AND DISCUSSION

3.1. Characteristics of the effluent before treatment

The textile effluent sample exhibited the following characteristics before undergoing any treatment. The pH of the effluent was 7.04, indicating a slightly alkaline nature. The Total Dissolved Solids (TDS) level was 221.7 mg/L, which is notably high and suggests a significant presence of dissolved substances that can affect water quality. The Electrical Conductivity (EC) was measured at 0.245 mS/cm, reflecting the effluent's ability to conduct electricity due to dissolved inorganic materials. Additionally, the Dissolved Oxygen (DO) content was 2.90 mg/L, a low value that indicates insufficient oxygen levels to support aerobic life forms effectively.

Heavy metal analysis revealed the presence of several toxic metals in the effluent. The concentrations were as follows: Chromium (Cr) at less than 0.9 ppm, Cadmium (Cd) at less than 0.8 ppm, Lead (Pb) at less than 1.06 ppm, Zinc (Zn) at less than 0.7 ppm, and Iron (Fe) at less than 0.9145 ppm. The levels of Lead, Chromium and Cadmium were particularly concerning as they exceeded acceptable limits, posing serious environmental and health risks.

Table 3. PhysicochemicalCharacteristicsof The Effluent

Parameters	Effluent
pH	7.04
TDS	221.7 mg/L
EC	0.245 mS/cm

DO	2.90 mg/L

Table 4. Heavy metal concentration of the effluent

Heavy Metals	Effluent
Cr	<0.9 ppm
Cd	<0.8 ppm
Pb	<1.06 ppm
Zn	<0.7 ppm
Fe	<0.9145 ppm

3.2. Physicochemical characters of theeffluent after treatment

The treated samples were analyzed for pH, TDS, EC, DO, and COD after treatments. These parameters have shown significant changes after various interventions. Improvement of the effluent after different treatments has been summarized in Table 5.

Table 5. Analysis of changes in physicochemical parameters of the samples throughout the treatments

Parameters	Treatments	Days				
		5	10	15	20	25
pH	T_{CV}	7.34	7.94	7.12	7.20	7.28
	T_{SP}	7.26	7.38	5.96	6.99	7.38
	T_{C}	7.38	7.75	7.22	7.15	7.21
TDS (mg/L)	T_{CV}	7750	455	8760	8040	6750
	T_{SP}	7250	8120	8320	6960	7310
	T_{C}	7070	792	8250	7250	7200
EC (mS/cm)	T_{CV}	15.49	0.913	17.43	16.05	13.48
	T_{SP}	14.49	16.24	16.62	13.93	15.68

	T_C	14.12	1.786	16.51	14.23	14.43
DO (mg/L)	T_{CV}	1.36	8.79	1.30	1.37	1.41
	T_{SP}	1.42	1.44	1.36	1.47	1.35
	T_C	1.39	8.99	1.42	1.44	1.40
COD (mg/L)	T_{CV}	519	488	413	370	301
	T_{SP}	511	473	404	355	295
	T_C	490	409	390	302	269

3.2.1. Changes on pH level

For the T_{CV} treatment, pH started at 7.34, peaked at 7.94 on Day 10, then decreased to 7.12 on Day 15 before stabilizing at 7.28 by Day 25. This moderate fluctuation suggests the treatment maintained a slightly alkaline range overall. In the T_{SP} treatment, the pH began at 7.26 and rose to 7.38 on Day 10. However, it significantly dropped to an acidic 5.96 on Day 15, indicating a drastic change likely due to the treatment process. By Day 20, the pH recovered to 6.99 and stabilized at 7.38 by Day 25, returning to a neutral state. The T_C treatment showed less variability, with pH starting at 7.38, increasing to 7.75 on Day 10, then gradually decreasing to 7.15 by Day 20, and stabilizing around 7.21 by Day 25, as observed in Fig. 4.

The T_{CV} and T_C treatments maintained pH The return to near-neutral pH levels by Day 25 across all treatments indicates the effluent's buffering capacity, which helps mitigate sudden pH changes and reduces environmental risks.

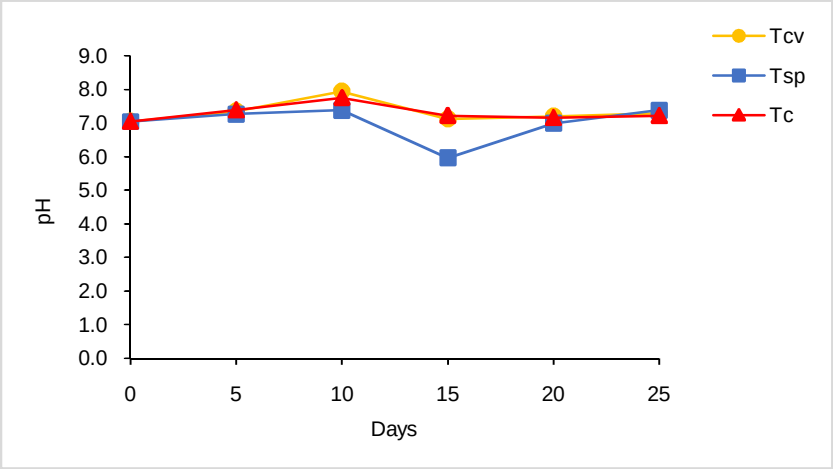

Fig. 4. Trend of change in pH level in different treatments

3.2.2. Changes on total dissolved solids (TDS)

For the T_{CV} treatment, TDS started at 7750 mg/L, dropped significantly to 455 mg/L by Day 10, spiked to 8760 mg/L on Day 15, then decreased to 8040 mg/L on Day 20, and finally reduced to 6750 mg/L by Day 25. This indicates substantial fluctuations throughout the treatment period. In the T_{SP} treatment, TDS started at 7250 mg/L, increased to 8120 mg/L on Day 10, and remained high, reaching 8320 mg/L on Day 15. It then decreased to 6960 mg/L by Day 20 and slightly increased to 7310 mg/L by Day 25. This treatment showed a generally high TDS level with some fluctuations. For the T_C treatment, TDS began at 7070 mg/L, dropped to 792 mg/L on Day 10, increased again to 8250 mg/L by Day 15, then decreased to 7250 mg/L on Day 20, and stabilized at 7200 mg/L by Day 25, as observed in Fig. 5.

T_{CV} saw a drop to 455 mg/L by Day 10 followed by a spike to 8760 mg/L on Day 15. T_C showed similar trends, with TDS dropping to 792 mg/L by Day 10 and spiking to 8250 mg/L on Day 15. The T_{SP} treatment maintained consistently high TDS levels throughout the period, suggesting it was less effective in reducing dissolved solids compared to the other treatments. Despite the

fluctuations, TDS levels in all treatments showed a trend towards stabilization by Day 25, which indicates that the treatment processes were starting to achieve a more balanced state. The substantial fluctuations in treatments highlight the need for improved control measures to ensure a more consistent reduction in TDS levels.

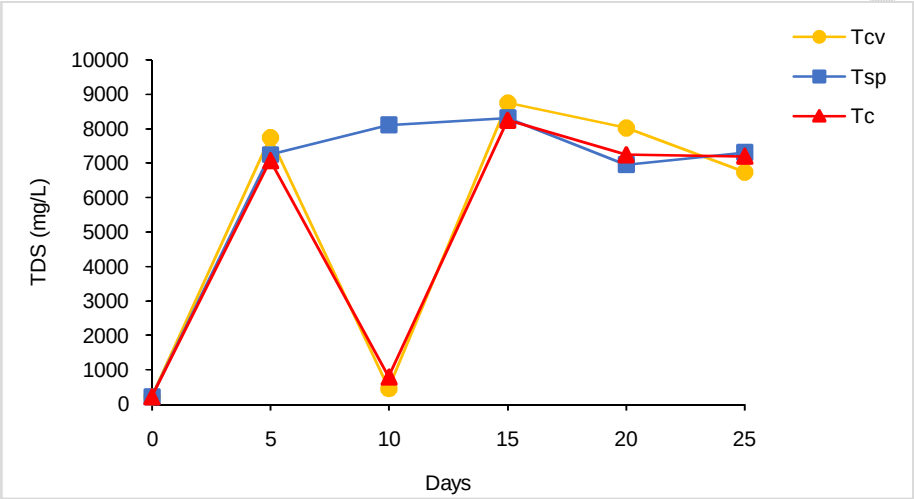

Fig. 5. Trend of change in total dissolved solids in different treatments

3.2.3. Changes on electrical conductivity (EC)

For the T_{CV} treatment, EC started at 15.49 mS/cm, dropped significantly to 0.913 mS/cm by Day 10, spiked to 17.43 mS/cm on Day 15, decreased to 16.05 mS/cm by Day 20, and further reduced to 13.48 mS/cm by Day 25. This indicates substantial fluctuations in EC throughout the treatment period. In the T_{SP} treatment, EC began at 14.49 mS/cm, increased to 16.24 mS/cm by Day 10, remained high, reaching 16.62 mS/cm on Day 15, then decreased to 13.93 mS/cm by Day 20, and slightly increased to 15.68 mS/cm by Day 25. This treatment maintained generally high EC levels with some fluctuations. For the T_C treatment, EC started at 14.12 mS/cm, dropped to 1.786 mS/cm by Day 10, increased again to 16.51 mS/cm by Day 15, then decreased to 14.23

mS/cm by Day 20, and stabilized at 14.43 mS/cm by Day 25. This treatment exhibited significant fluctuations but showed some stabilization towards the end, as observed in Fig. 6.

The T_{SP} treatment maintained consistently high EC levels throughout the period, suggesting it was less effective in reducing dissolved ionic substances compared to the other treatments. Despite the fluctuations, EC levels in all treatments showed a trend towards stabilization by Day 25, indicating that the treatment processes were starting to achieve a more balanced state. High EC levels reflect the presence of dissolved salts and inorganic materials in the effluent, which can affect water quality and treatment processes. The substantial fluctuations in treatments highlight the need for improved control measures to ensure a more consistent reduction in EC levels.

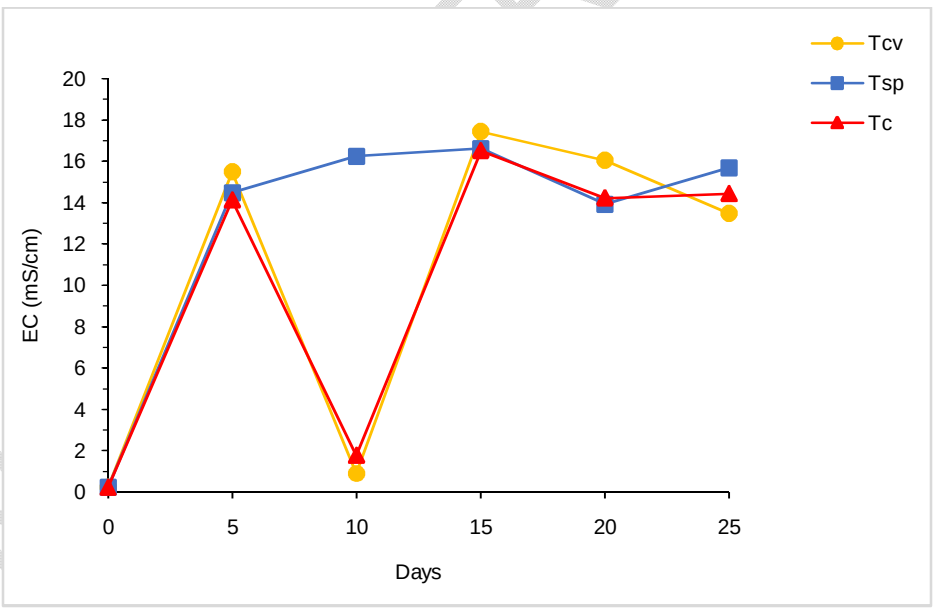

Fig. 6. Trend of change in electrical conductivity in different treatments

3.2.4. Changes on the level of dissolved oxygen (DO)

For the T_{CV} treatment, DO started at 1.36 mg/L, increased significantly to 8.79 mg/L by Day 10, dropped to 1.30 mg/L on Day 15, then slightly increased to 1.37 mg/L by Day 20, and further stabilized at 1.41 mg/L by Day 25. This indicates substantial fluctuations in DO throughout the treatment period. In the T_{SP} treatment, DO began at 1.42 mg/L, increased marginally to 1.44 mg/L by Day 10, slightly decreased to 1.36 mg/L on Day 15, rose to 1.47 mg/L by Day 20, and finally dropped slightly to 1.35 mg/L by Day 25. This treatment maintained relatively stable but low DO levels. For the T_C treatment, DO started at 1.39 mg/L, increased significantly to 8.99 mg/L by Day 10, dropped to 1.42 mg/L by Day 15, then slightly increased to 1.44 mg/L by Day 20, and stabilized at 1.40 mg/L by Day 25. This treatment also exhibited significant fluctuations but showed some stabilization towards the end, as observed in Fig. 7.

T_{CV} saw a spike to 8.79 mg/L by Day 10, followed by a drop to 1.30 mg/L on Day 15. T_C showed similar trends, with DO spiking to 8.99 mg/L by Day 10 and dropping to 1.42 mg/L by Day 15. The T_{SP} treatment maintained relatively stable but low DO levels throughout the period, suggesting it was less effective in increasing oxygen levels in the effluent. Despite the fluctuations, DO levels in all treatments showed a trend towards stabilization by Day 25, indicating that the treatment processes were starting to achieve a more balanced state.

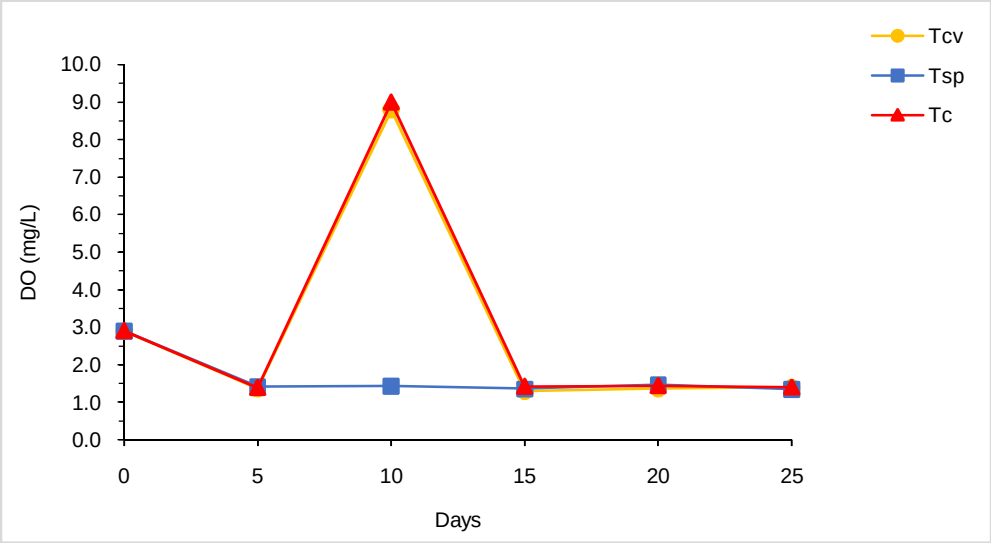

Fig. 7. Trend of change in dissolved O_2 level in different treatments

3.2.5. Changes on the level of chemical oxygen demand (COD)

For the T_{CV} treatment, COD started at 519 mg/L, decreased to 488 mg/L by Day 10, continued to drop to 413 mg/L on Day 15, further reduced to 370 mg/L by Day 20, and finally lowered to 301 mg/L by Day 25. This indicates a consistent reduction in COD throughout the treatment period. In the T_{SP} treatment, COD began at 511 mg/L, decreased to 473 mg/L by Day 10, dropped further to 404 mg/L on Day 15, continued to decrease to 355 mg/L by Day 20, and finally reduced to 295 mg/L by Day 25. This treatment showed a steady decline in COD levels over time. For the T_C treatment, COD started at 490 mg/L, decreased to 409 mg/L by Day 10, continued to drop to 390 mg/L on Day 15, further reduced to 302 mg/L by Day 20, and stabilized at 269 mg/L by Day 25. This treatment exhibited a significant and consistent reduction in COD levels, as observed in Fig. 8.

All treatments showed a consistent and significant reduction in COD levels over the 25-day period. T_{CV} saw a decrease from 519 mg/L to 301 mg/L, T_{SP} from 511 mg/L to 295 mg/L, and T_C from 490 mg/L to 269 mg/L. This indicates that the treatments were effective in reducing the

organic load in the effluent. The steady decline in COD levels suggests that the treatment processes were efficient in breaking down organic compounds and reducing the effluent's overall oxygen demand. By Day 25, all treatments showed stabilization of COD levels, indicating that the treatment processes had reached a more balanced and effective state. The consistent reduction and stabilization of COD across all treatments highlight the effectiveness of the treatment processes in improving effluent quality, making it safer for discharge into the environment.

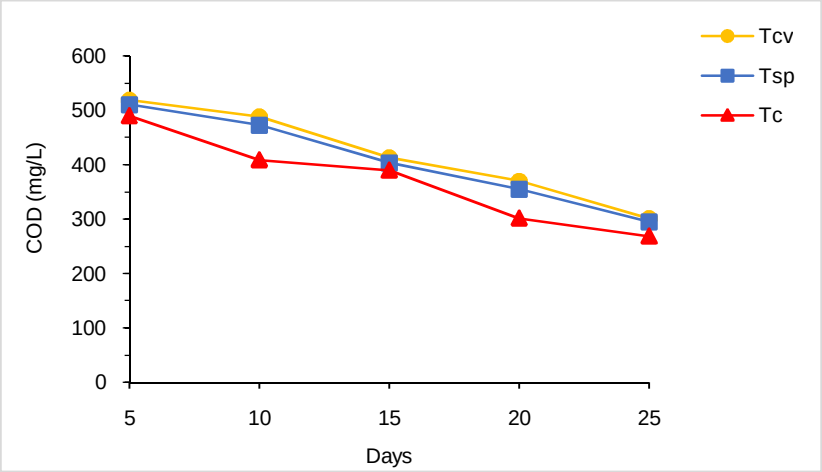

Fig. 8. Trend of change in chemical O_2 demand in different treatments

3.3. Heavy metal concentration of the effluent after treatment

Heavy metals were analyzed in both treated and untreated effluent by acid-digesting all the samples. Collected textile wastewater carries Cr, Cd, Pb, Zn, and Fe. Changes is heavy metal quantities after treatments are summarized in Table 6.

Table 6. Analysis of heavy metal conc. of the effluent throughout the treatment

Heavy metals (ppm) Treatments	Days				
	5	10	15	20	25

Cr	T_{CV}	0.783	0.702	0.651	0.581	0.462
	T_{SP}	0.774	0.701	0.677	0.602	0.584
	T_C	0.874	0.705	0.633	0.596	0.501
Cd	T_{CV}	0.721	0.688	0.601	0.552	0.473
	T_{SP}	0.722	0.702	0.652	0.549	0.533
	T_C	0.779	0.701	0.654	0.593	0.506
Pb	T_{CV}	0.891	0.782	0.721	0.674	0.553
	T_{SP}	0.904	0.851	0.750	0.679	0.605
	T_C	1.010	0.901	0.854	0.755	0.673
Zn	T_{CV}	0.552	0.501	0.441	0.391	0.301
	T_{SP}	0.691	0.632	0.602	0.599	0.532
	T_C	0.681	0.605	0.567	0.501	0.473
Fe	T_{CV}	0.788	0.701	0.681	0.601	0.501
	T_{SP}	0.879	0.805	0.776	0.707	0.632
	T_C	0.867	0.779	0.703	0.679	0.603

3.3.1. Impact overCrConcentration

For the T_{CV} treatment, the concentration of Cr started at 0.783 ppm, decreased gradually to 0.462 ppm by Day 25. This treatment showed a consistent reduction in Cr concentration over the treatment period. In the T_{SP} treatment, the concentration of Cr started at 0.774 ppm, decreased to 0.584 ppm by Day 25. Similar to T_{CV}, this treatment exhibited a consistent decline in Cr concentration over time. For the T_C treatment, the concentration of Cr started at 0.874 ppm, decreased to 0.501 ppm by Day 25. This treatment also showed a steady reduction in Cr concentration throughout the treatment period, as observed in Fig. 9.

All treatments showed a consistent and significant decrease in Cr concentration over the 25-day period. The steady decline in Cr concentration suggests that the treatment processes were efficient in removing chromium from the effluent. By Day 25, all treatments showed stabilization of Cr concentration, indicating that the treatment processes had reached a more balanced and effective state. The consistent reduction in Cr concentration observed across all

treatments demonstrates the effectiveness of the treatment processes in improving effluent quality and reducing the potential risks associated with chromium contamination.

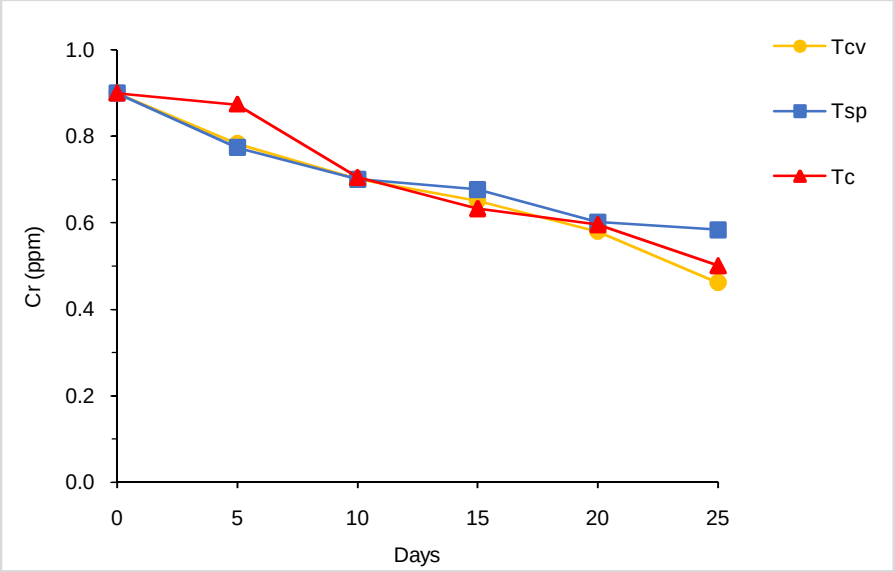

Fig. 9. Differences in the Cr conc. pre and post treatments

3.3.2. Impact over Cd Concentration

For the T_{CV} treatment, the concentration of Cd started at 0.721 ppm, decreasing gradually to 0.473 ppm by Day 25. This treatment showed a consistent reduction in Cd concentration over the treatment period.In the T_{SP} treatment, the concentration of Cd started at 0.722 ppm, decreased to 0.533 ppm by Day 25. Similar to T_{CV}, this treatment exhibited a consistent decline in Cd concentration over time.For the T_C treatment, the concentration of Cd started at 0.779 ppm, decreased to 0.506 ppm by Day 25. This treatment also showed a steady reduction in Cd concentration throughout the treatment period, as observed in Fig. 10.

All treatments showed a consistent and significant decrease in Cd concentration over the 25-day period. The steady decline in Cd concentration suggests that the treatment processes were efficient in removing cadmium from the effluent. By Day 25, all treatments showed stabilization

of Cd concentration, indicating that the treatment processes had reached a more balanced and effective state.The consistent reduction in Cd concentration observed across all treatments demonstrates the effectiveness of the treatment processes in improving effluent quality and reducing the potential risks associated with cadmium contamination.

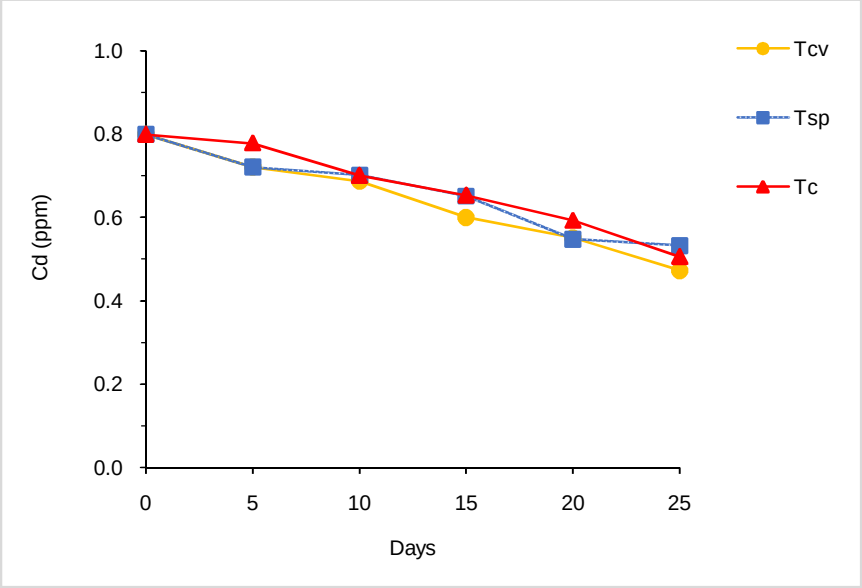

Fig. 10. Differences in the Cd conc. pre and post treatments

3.3.3. Impact over Pb Concentration

For the T_{CV} treatment, the concentration of Pb started at 0.891 ppm, decreased gradually to 0.553 ppm by Day 25. This treatment showed a consistent reduction in Pb concentration over the treatment period. In the T_{SP} treatment, the concentration of Pb started at 0.904 ppm, decreased to 0.605 ppm by Day 25. Similar to T_{CV}, this treatment exhibited a consistent decline in Pb concentration over time. For the T_C treatment, the concentration of Pb started at 1.010 ppm, decreased to 0.673 ppm by Day 25. This treatment also showed a steady reduction in Pb concentration throughout the treatment period, as observed in Fig. 11.

All treatments showed a consistent and significant decrease in Pb concentration over the 25-day period. The steady decline in Pb concentration suggests that the treatment processes were efficient in removing lead from the effluent. By Day 25, all treatments showed stabilization of Pb concentration, indicating that the treatment processes had reached a more balanced and effective state. The consistent reduction in Pb concentration observed across all treatments demonstrates the effectiveness of the treatment processes in improving effluent quality and reducing the potential risks associated with lead contamination.

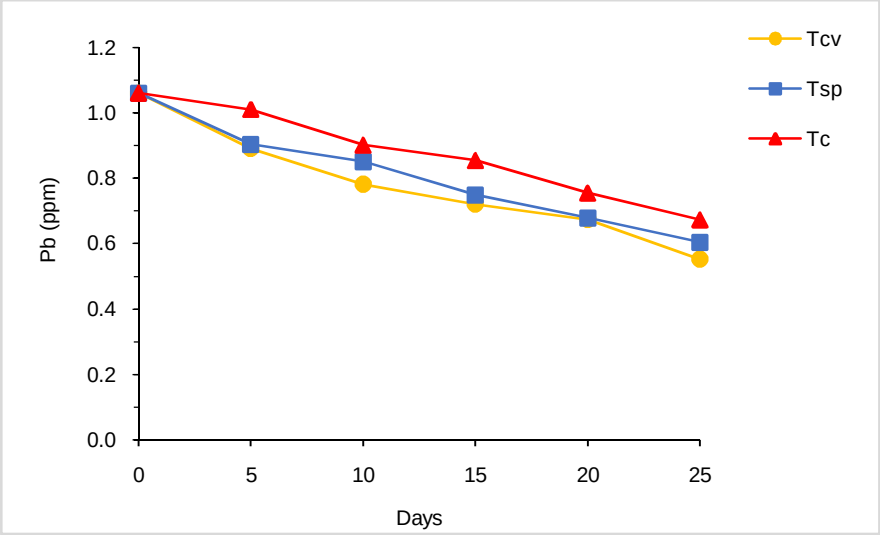

Fig. 11. Differences in the Pb conc. pre and post treatments

3.3.4. Impact over Zn Concentration

For the T_{CV} treatment, the concentration of Zn started at 0.552 ppm, decreasing gradually to 0.301 ppm by Day 25. This treatment showed a consistent reduction in Zn concentration over the treatment period. In the T_{SP} treatment, the concentration of Zn started at 0.691 ppm, decreased to 0.532 ppm by Day 25. Similar to T_{CV}, this treatment exhibited a consistent decline in Zn concentration over time. For the T_C treatment, the concentration of Zn started at 0.681 ppm,

decreased to 0.473 ppm by Day 25. This treatment also showed a steady reduction in Zn concentration throughout the treatment period, as observed in Fig. 12.

All treatments showed a consistent and significant decrease in Zn concentration over the 25-day period. The steady decline in Zn concentration suggests that the treatment processes were efficient in removing zinc from the effluent. By Day 25, all treatments showed stabilization of Zn concentration, indicating that the treatment processes had reached a more balanced and effective state. The consistent reduction in Zn concentration observed across all treatments demonstrates the effectiveness of the treatment processes in improving effluent quality and reducing the potential risks associated with zinc contamination.

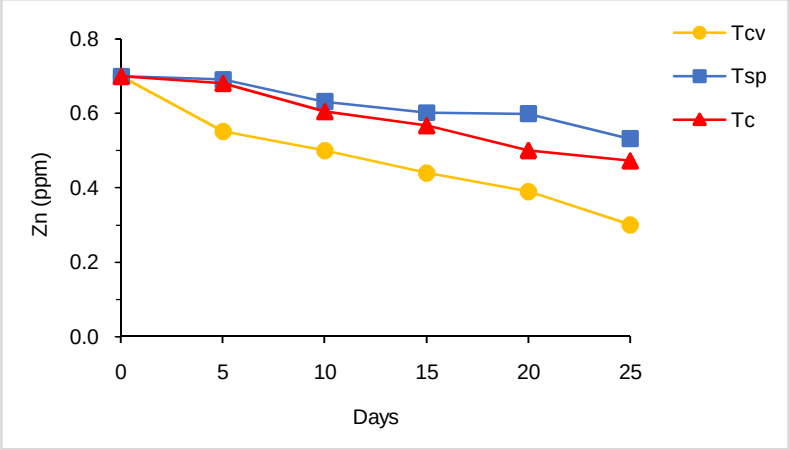

Fig. 12. Differences in the Zn conc. pre and post treatments

3.3.5. Impact over Fe Concentration

For the T_{CV} treatment, the concentration of Fe started at 0.788 ppm, decreasing gradually to 0.501 ppm by Day 25. This treatment showed a consistent reduction in Fe concentration over the treatment period. In the T_{SP} treatment, the concentration of Fe started at 0.879 ppm, decreased to 0.632 ppm by Day 25. Similar to T_{CV}, this treatment exhibited a consistent decline in Fe concentration over time. For the T_C treatment, the concentration of Fe started at 0.867 ppm,

decreased to 0.603 ppm by Day 25. This treatment also showed a steady reduction in Fe concentration throughout the treatment period, as observed in Fig. 13.

All treatments showed a consistent and significant decrease in Fe concentration over the 25-day period. The steady decline in Fe concentration suggests that the treatment processes were efficient in removing iron from the effluent. By Day 25, all treatments showed stabilization of Fe concentration, indicating that the treatment processes had reached a more balanced and effective state. Excessive concentrations can lead to promoting the growth of iron-oxidizing bacteria, which can disrupt aquatic habitats. The consistent reduction in Fe concentration observed across all treatments demonstrates the effectiveness of the treatment processes in improving effluent quality.

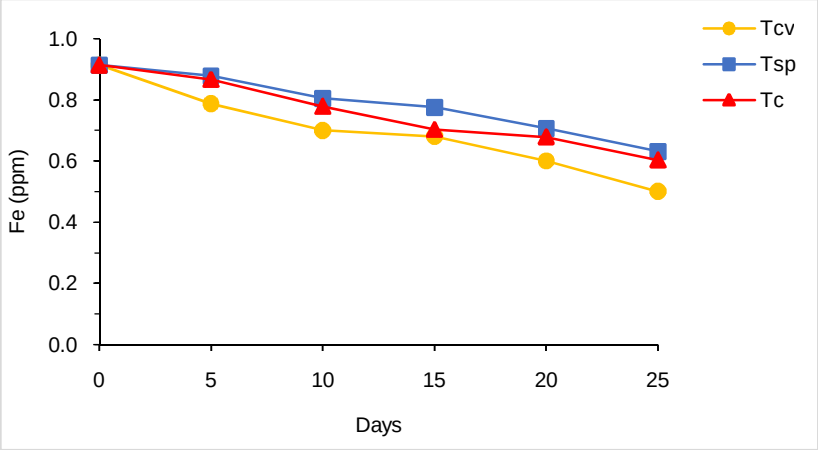

Fig. 13. Differences in the Fe conc. pre and post treatments

4. CONCLUSION

Our study aimed to improve textile wastewater effluent by optimizing its physicochemical properties and reducing heavy metal concentrations through biological treatments using *Spirulina* sp. and *Chlorella* sp. treatments. The results demonstrated significant improvements in effluent quality across all treatments over the 25-day period, which effectively restored the

effluent's pH to near-neutral levels, mitigating potential environmental risks associated with acidic or alkaline conditions. Additionally, substantial reductions in TDS, EC, and COD were observed, indicating the treatments' efficiency in removing dissolved contaminants and organic matter from the effluent. Moreover, the treatments successfully lowered the concentrations of toxic heavy metals such as chromium (Cr), cadmium (Cd), lead (Pb), zinc (Zn), and iron (Fe) to levels below permissible limits, thereby reducing the environmental and health risks associated with heavy metal contamination.

Overall, the results suggest that biological treatments using *Spirulina platensis* and *Chlorella vulgaris* treatments have significant potential for improving textile wastewater effluent quality and reducing heavy metal pollution. Future research could focus on optimizing treatment conditions, exploring the mechanisms underlying the remediation processes, and assessing the long-term environmental impacts of these treatments. Additionally, further investigations into the potential use of other microalgae species and combinations of treatments could provide valuable insights into enhancing effluent treatment efficiency and sustainability.

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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.

AUTHORS' CONTRIBUTIONS

NNH: Conceptualization, Methodology, Data curation, Writing- Original Draft, Writing- Review & Editing, Supervision, Funding Acquisition; MAA: Investigation, Software, ASB: Data curation, Writing- Review & Editing, Visualization; EAZ: Methodology, Resources; CKR: Writing- Review & Editing; MKH: Visualization, Formal Analysis and JLM: Supervision, Project Administration, Funding Acquisition.

DATA AVAILABILITY

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

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