



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

**Natasha Nafisa Haque ^{a*}, Md. Ashrafal Alam ^b,
Anthony Samit Baidya ^a, Elina Akther Zenat ^a,
Chapol Kumar Roy ^a, Md. Kamal Hossain ^a
and John Liton Munshi ^{a*}**

^a *Biological Research Division, BCSIR Dhaka Laboratories, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh.*

^b *Institute of Glass and Ceramic Research and Testing (IGCRT), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh.*

Authors' contributions

This work was carried out in collaboration among all authors. Author NNH conceptualized, methodology, data curation, wrote the original draft and wrote reviewed and edited the manuscript, supervision, funding acquisition. Author MAA did Investigated, Software. Author ASB Data curation, wrote original draft, reviewed and edited the manuscript. Author EAZ methodology, resources. Author CKR did visualized, wrote reviewed and edited the manuscript. Author MKH visualized, formal analyzed. Author JLM supervision, project administrated, funding acquisition. All authors read and approved the final manuscript.

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*Corresponding author: Email: nafisahaque@bcsir.gov.bd, johnliton@bcsir.gov.bd;

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ABSTRACT

Aims: The study aimed 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 characteristics (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 with 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 using physicochemical parameters and heavy metal concentration before 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 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 intervals till the 25th 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 Narayanganj, 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 effects which can be acute, chronic, sub-chronic, neurotoxic, carcinogenic, mutagenic or teratogenic [5-8]. 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 [9]. 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 [10]. Lead poisoning can cause inhibition of hemoglobin synthesis, kidney and joint dysfunctions, reproductive system issues, and acute and chronic damage to the central nervous system [11]. 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 [12,13]. Recent studies have focused on the adsorptive removal of heavy metals and dyes using chitosan-based materials [14,15].

Four common ways to treat wastewater include physical water treatment, biological water treatment, chemical treatment and sludge treatment. Agents used for bioremediation are bacteria, fungi and algae [16]. 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 [17]. 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 [18]. 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 [18]. 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 [19].

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. [20]. It is also characterized by a higher capacity to remove effluents from textile wastewater [21]. 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 [22]. Many studies examined the impact [23-37] of various dyes on water, concluding that higher dye concentrations inhibit the growth of *Spirulina* sp. and reduce its nutrient levels [38].

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 [39]. With the country's population rising, the demand for landfills to relocate polluted material surpasses the available supply [40]. On the other hand, biological treatment could achieve greater efficiencies in the decolourization and detoxification of textile wastewater by using native aquatic plants [41-43]. Using microorganisms to break down pollutants or waste, such as oil spills, contaminated groundwater or industrial processes makes bioremediation a very attractive solution [23].

This study focused on the growth parameter optimization of *Spirulina* sp. 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 AND METHODS

2.1 Textile Effluent Collection

Textile wastewater was collected from the textile mills in Narayanganj, 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 anaerobic conditions and mixed vertically. The samples were not stratified concerning pH, temperature or electrical conductivity. The water also had a dark-brown colour.

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 samples were collected (in fresh plastic gallons). 5.0 mL of conc. HNO₃ 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. 1), provided the cyanobacteria *Spirulina platensis* and *Chlorella vulgaris*.

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 agitated over an orbital shaker (SYC-2102) and exposed to white light for a 24.0 hours photoperiod by using a cool white fluorescent light. Cell growth was monitored by determination of optical density (OD₇₅₀) at 750.0 nm. For *Spirulina* culture, Zarrouk's medium of pH 9.50 (Table 1) was used and for *Chlorella* culture, CH medium was used (Table 2). The Applied Botany Laboratory, BCSIR Dhaka Laboratories, provided the reagents for media preparation.

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 (OD₆₀₀, 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.

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	16.80
K ₂ HPO ₄	0.50
MgSO ₄ .7H ₂ O	0.20
FeSO ₄ .7H ₂ O	0.010
CaCl ₂ .2H ₂ O	0.040
EDTA-Na ₂ .2H ₂ O	0.080
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

Table 2. Composition of CH medium

Chemicals	Amount (g/L)
KNO ₃	6.000
K ₂ HPO ₄	0.240
MgSO ₄	0.060
FeSO ₄	0.030
CaSO ₄ .2H ₂ O	0.012

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.

2.7 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 Hach SensION 4-Thermo Fisher.

2.8 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 Hach SensION 4-Thermo Fisher provides a direct measurement of salinity.

2.9 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 Hach SensION 4-Thermo Fisher.

2.10 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 Hach SensION 4-Thermo Fisher.

2.11 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.

2.12 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 H₂O = 1:9) was prepared. For each reaction, 10.0 mL of the prepared sample was mixed with 5.0 mL of K₂Cr₂O₇ solution (0.25 N), 15.0 mL of AgSO₄-H₂SO₄ solution (10.0 mg/mL), and 0.02 g HgSO₄ 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 H₂O. Next, eight (8) drops of Ferroin indicator were added to the mixture and it was titrated against standard FeSO₄·(NH₄)₂SO₄·6H₂O solution (0.25 N) until the blue-green colour changes to red wine. The procedure was also carried out for the blank sample.



Fig.1. *Spirulina platensis* raceway pond at BCSIR Laboratories, Dhaka

2.13 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 HNO₃, and 5.0 – 10.0 ml of H₂O₂ 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 "AAAnalyst 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/cm², 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. Physicochemical characteristics of the effluent

Parameters	Effluent
pH	7.04
TDS	221.70 mg/L
EC	0.245 mS/cm
DO	2.90 mg/L

Table 4. Heavy metal concentration of the effluent

Heavy Metals	Effluent
Cr	< 0.90 ppm
Cd	< 0.80 ppm
Pb	< 1.06 ppm
Zn	< 0.70 ppm
Fe	< 0.91450 ppm

3.2 Physicochemical Characters of the Effluent 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.

3.3 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. 2.

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.

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

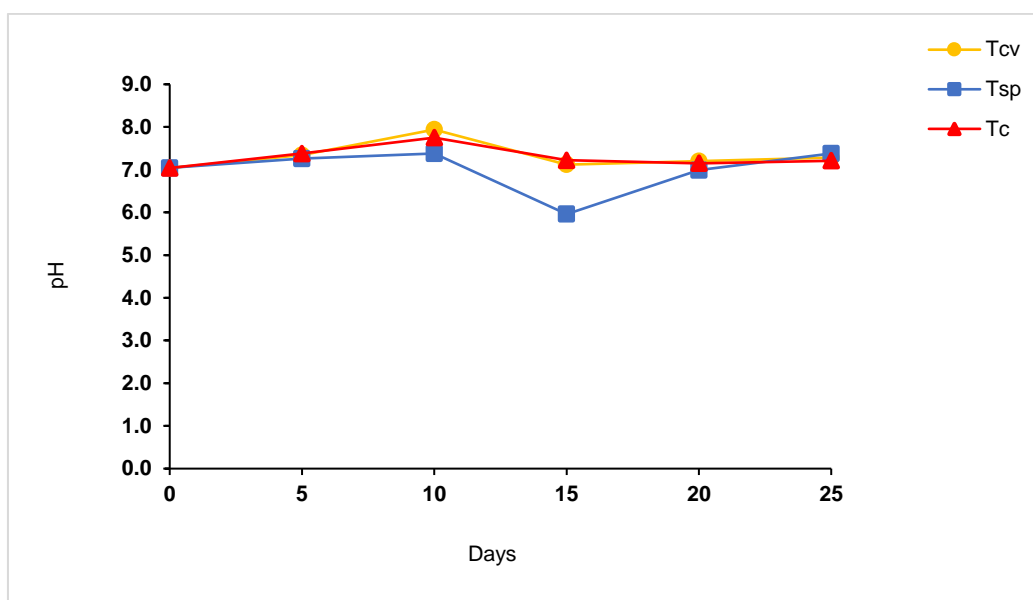


Fig. 2. Trend of change in pH level in different treatments

3.4 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. 3.

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.

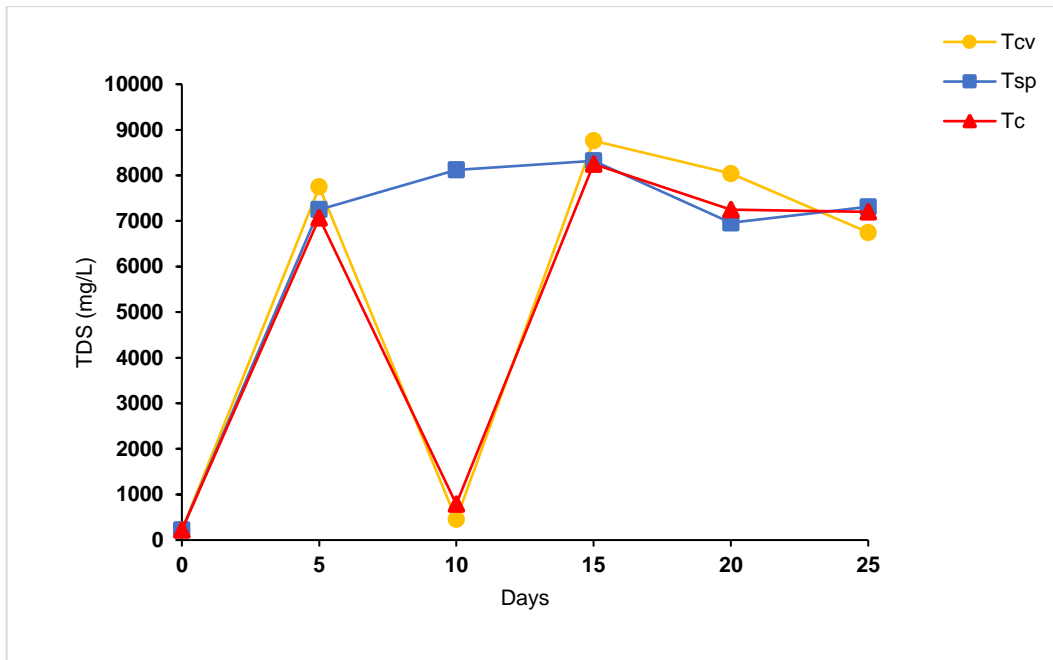


Fig. 3. Trend of change in total dissolved solids in different treatments

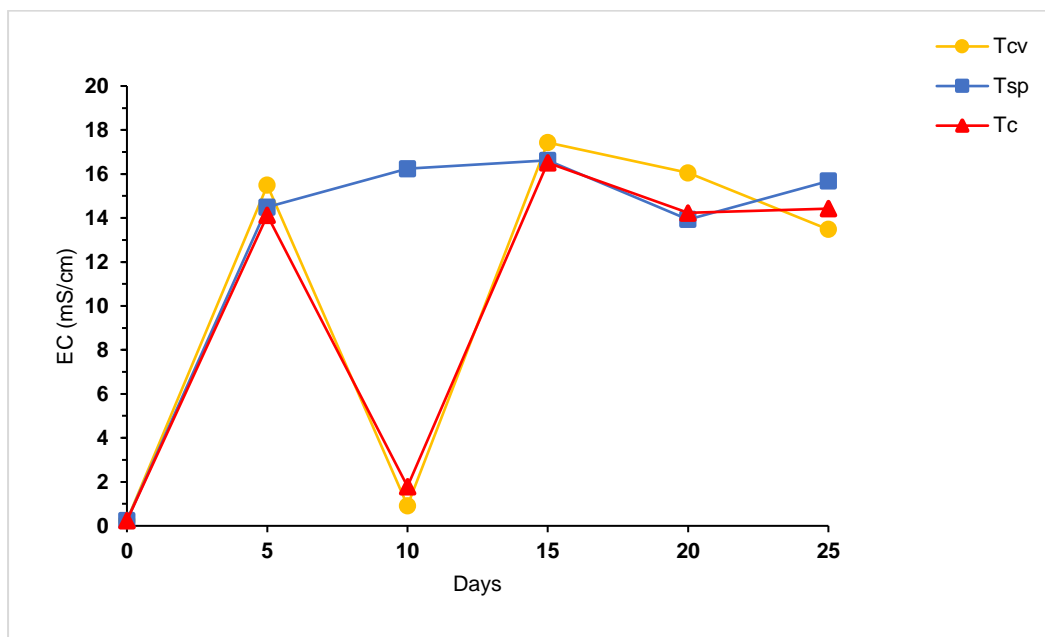


Fig. 4. Trend of change in electrical conductivity in different treatments

3.5 Changes in 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. 4.

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.

3.6 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. 5.

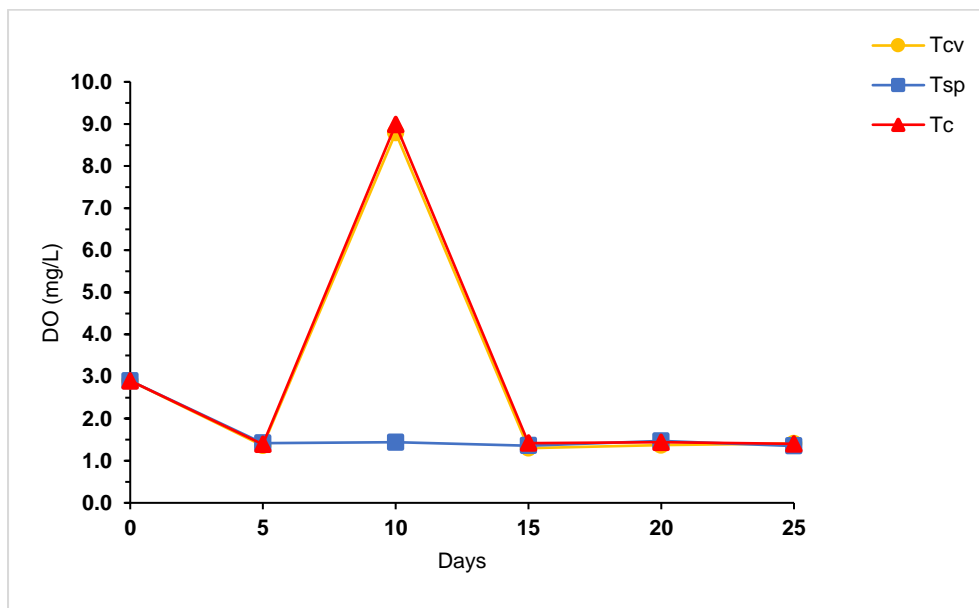


Fig. 5. Trend of change in dissolved O₂ level in different treatments

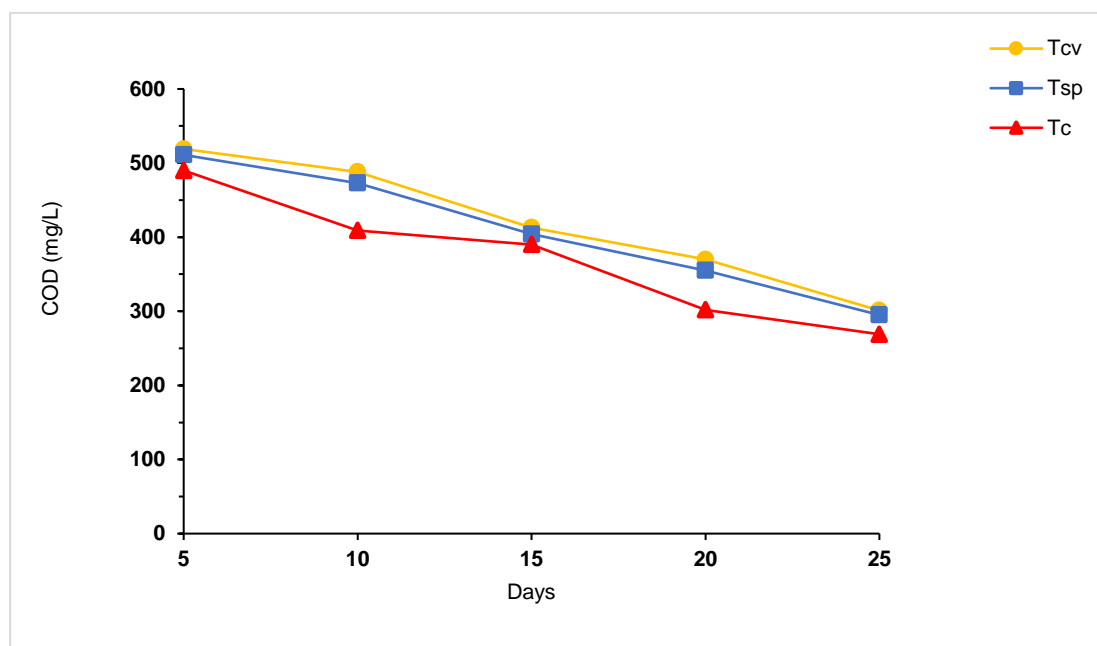


Fig. 6. Trend of change in chemical O₂ demand in different treatments

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.

3.7 Changes in 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. 6.

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.

3.8 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 in 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

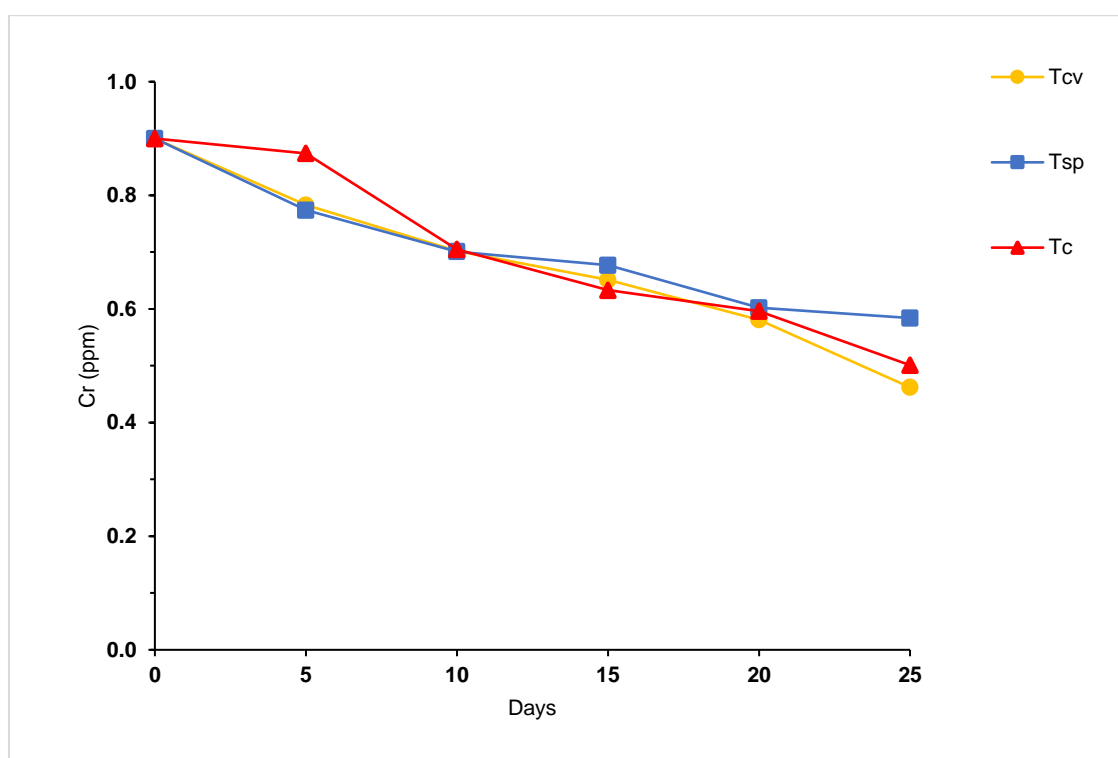


Fig. 7. Differences in the Cr conc. pre and post treatments

3.9 Impact over Cr Concentration

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. 7.

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.

3.10 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, and

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 and 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. 8.

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.

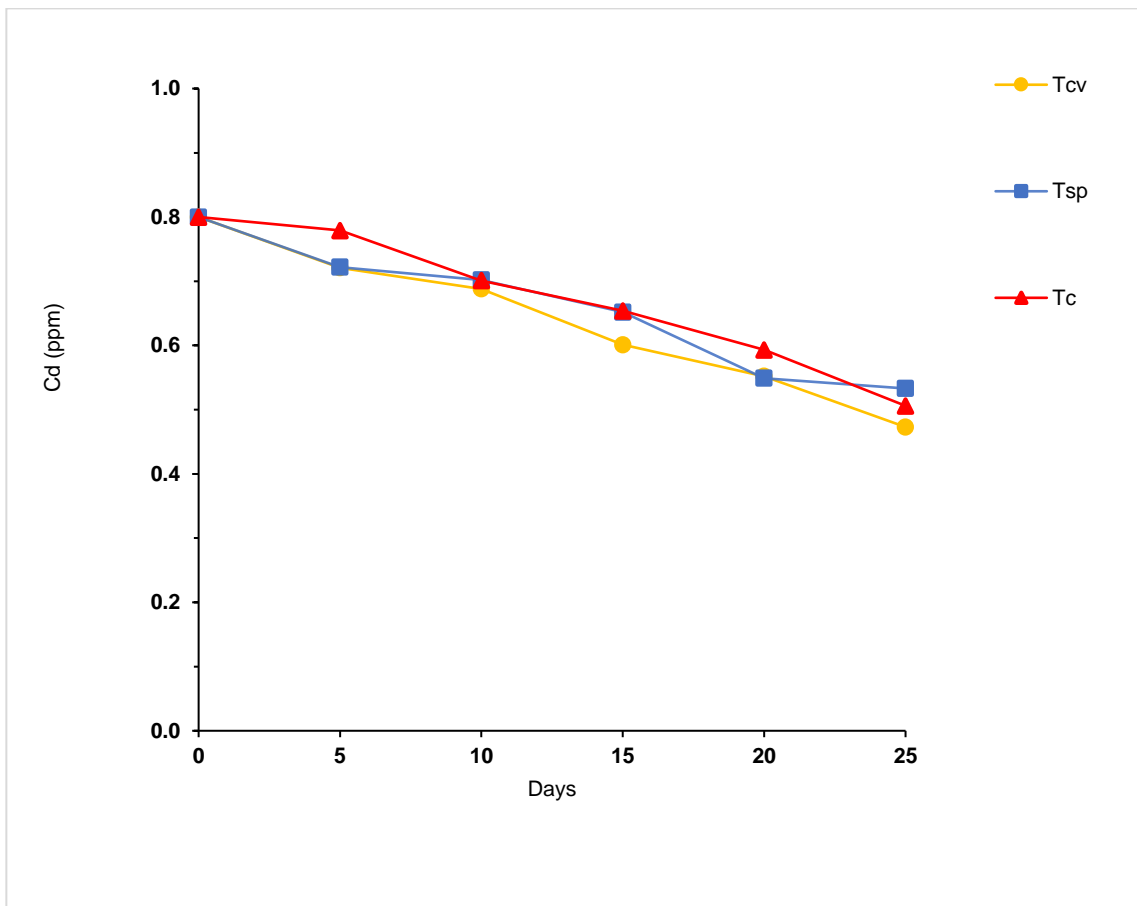


Fig. 8. Differences in the Cd conc. pre and post treatments

3.11 Impact over Pb Concentration

For the T_{CV} treatment, the concentration of Pb started at 0.891 ppm and 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 and 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 and 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. 9.

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.

3.12 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 and 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 and 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. 10.

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.

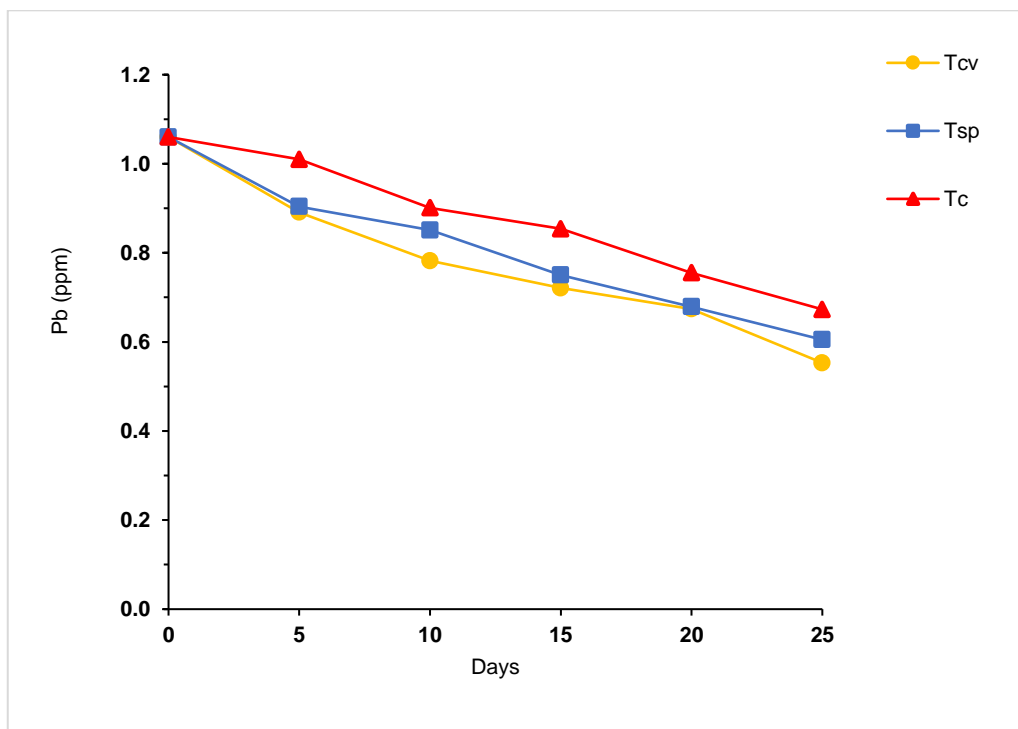


Fig. 9. Differences in the Pb conc. pre and post treatments

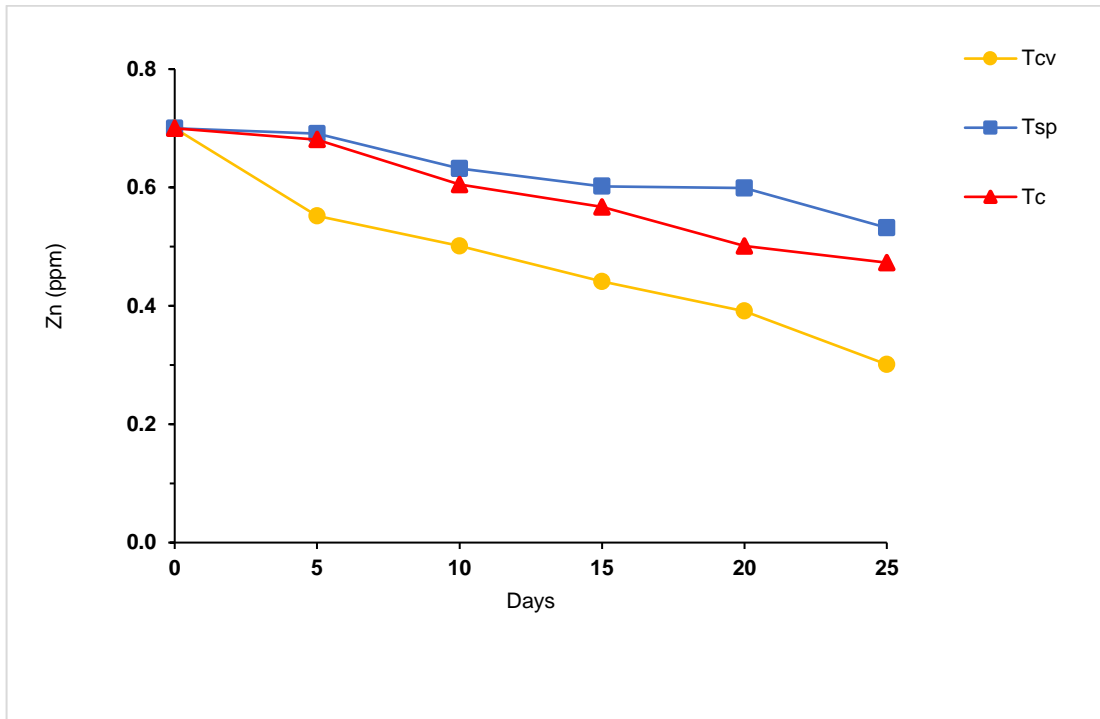


Fig. 10. Differences in the Zn conc. pre and post treatments

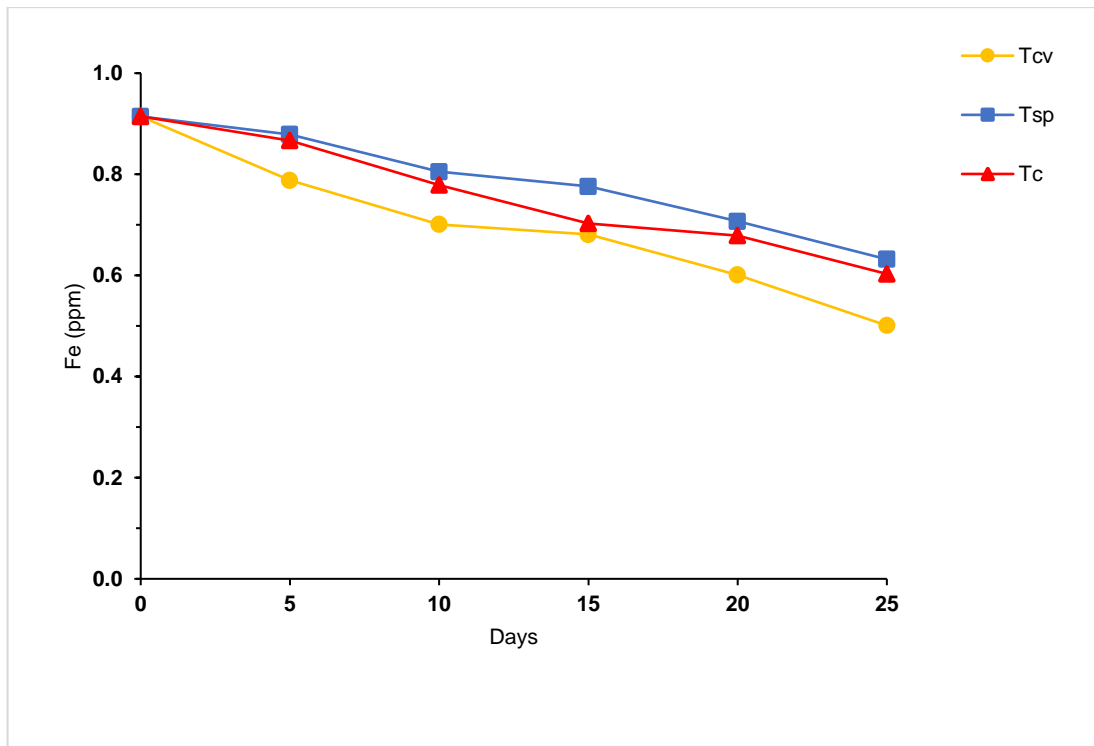


Fig. 11. Differences in the Fe conc. pre and post treatments

3.13 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 and 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 and 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. 11.

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.

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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of manuscripts.

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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

REFERENCES

1. Alvarez EA, Mochon MC, Sánchez JJ, Rodríguez MT, Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere*. 2002;47(7):765-775.
2. Noor R, Ahmed T, Munshi SK. State of bioremediation in Bangladesh: current concept and implementation compared to global approaches. *CLEAN—Soil, Air, Water*. 2017;45(1).
3. Roy C., Jahan, M, Rahman S. Characterization and treatment of textile wastewater by aquatic plants (macrophytes) and algae. *Eur J Sustain Dev Res*. 2018;2(3):29.

4. Jiries AG, Al Nasir FM, Beese F. Pesticide and heavy metals residue in wastewater, soil and plants in wastewater disposal site near Al-Lajoun Valley, Karak/Jordan. *Water, Air, and Soil Pollution*. 2002;133(1):97-107.
5. Ramachandran P, Sundharam R, Palaniyappan J, Munusamy AP. Potential process implicated in bioremediation of textile effluents: a review. *Adv Appl Sci Res*. 2013;4(1):131-145.
6. Torres L, Lopez Y, Gomez-y-Gomez Y, Bautista E, J. Corzo L. Production and Broad Characterization of a *Spirulina platensis* Dry Powder Grown in Bubbled Columns. *J. Adv. Microbiol*. 20189(3):1-16. Accessed on:2024 May 24 Available:<https://journaljamb.com/index.php/JAMB/article/view/42>
7. Ogbonna DN, Origbe ME. Heavy Metal Concentration of Surface Water, Sediment and Fishes Impacted by Crude Oil Pollution in Bodo/Bonny River, Nigeria. *Curr. J. Appl. Sci. Technol*. 2021 ;40(18):77-8. Accessed On:2024 May 24 Available:<https://journalcjast.com/index.php/CJAST/article/view/3578>
8. Dhote J, Ingole S, Chavhan A. Review of wastewater treatment technologies. *International Journal of Engineering Research & Technology*. 2012;1(5):1-10.
9. Dixit R, Malaviya D, Pandiyan K, Singh UB, Sahu A, Shukla R, Singh BP, Rai JP, Sharma PK, Lade H, Paul D. Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. *Sustainability*. 2015;7(2):2189-2212.
10. Bayoumi MN, Al-Wasify RS, Hamed SR. Bioremediation of textile wastewater dyes using local bacterial isolates. *International Journal of Current Microbiology and Applied Sciences*. 2014;3(12):962-970.
11. da Silva Oliveira A, Bocio, A, Beltramini Trevilato TM, Magosso Takayanagui AM, Domingo JL, Segura-Muñoz SI. Heavy metals in untreated/treated urban effluent and sludge from a biological wastewater treatment plant. *Environmental Science and Pollution Research-International*. 2007;14(7):483-489.
12. Wang L, Min M, Li Y, Chen P, Chen Y, Liu Y, Wang Y, Ruan R. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology*. 2010;162(4):1174-1186.
13. Darwesh OM, Moawad H, El-Rahim WMA, Barakat OS, Sedik MZ. Bioremediation of textile reactive blue (RB) azo dye residues in wastewater using experimental prototype bioreactor. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2014;5(4):1203-1219.
14. Dwivedi P, Tomar RS. Bioremediation of textile effluent for degradation and decolorization of synthetic dyes: a review. *International Journal of Current Research in Life Sciences*. 2018;7(4):1948-1951.
15. Ali S, Rizwan M, Ibrahim M, Nafees M, Waseem M. Role of bioremediation agents (bacteria, fungi, and algae) in alleviating heavy metal toxicity. In *Probiotics in agroecosystem*. Springer, Singapore. 2017;517-537.
16. Salgueiro JL, Perez L, Maceiras R, Sanchez A, Cancela A, Bioremediation of wastewater using *Chlorella vulgaris* microalgae: Phosphorus and organic matter. *International Journal of Environmental Research*. 2016;10(3):465-470.
17. Jahan MAA, Akhtar N, Khan N. MS, Roy CK, Islam R, Nurunnabi M. Characterization of tannery wastewater and its treatment by aquatic macrophytes and algae. *Bangladesh Journal of Scientific and Industrial Research*. 2014;49 (4):233-242.
18. Lim SL, Chu WL, Phang SM. Use of *Chlorella vulgaris* for bioremediation of textile wastewater. *Bioresource Technology*. 2010;101(19):7314-7322.
19. Kulkarni SD, Auti T, Saraf S. Bioremediation study of dairy effluent by using *Spirulina platensis*. *Res. J. Life Sci. Bioinform. Pharm. Chem. Sci*. 2016; 1(6):325.
20. Abdel-Razek MA, Abozeid AM, Eltholth MM, Abouelenien FA, El-Midany SA, Moustafa NY, Mohamed RA. Bioremediation of a pesticide and selected heavy metals in wastewater from various sources using a consortium of microalgae and cyanobacteria. *Slov Vet*. 2019;56(Suppl 22):61-73.
21. Moradi Z, Haghjou MM, Zarei M, Colville L, Raza A. Synergy of production of value-added bioplastic, astaxanthin and phycobilin co-products and Direct Green 6 textile dye remediation in *Spirulina*

- platensis. *Chemosphere*. 2021;280; 130920.
22. Zenat M, Akther E, Haque NN, Hasan MR, Begum M, Munshi JL, Alam MA. Antifungal activity of various plant extracts against aspergillus and penicillium species isolated from leather-borne fungus. *Microbiology Research Journal International*. 2024;34(1):10-23.
 23. Haque NN, Alam MA, Roy CK, Zenat M, Akther E, Munshi JL. Cyanobacteria Mediated CO₂ Segregation: A Promising Alternative Method for Sustainable Bioremediation and Biomass Production. *Asian Journal of Research in Biochemistry*. 2023;13(3):28-43.
 24. Munshi JL, Baksha R, Rahaman MZ, Huque NN, Zinat EA, Momtaz N. *In Vitro* plant regeneration from leaf explants of *Tagetes erecta* L. *Bangladesh Journal of Scientific and Industrial Research*. 2021;56(2):69-74.
 25. Sachchu, Md. Mazedul Haque, Amir Hossain, Md. Mahmudul Kobir, Md. Durul Hoda, Md. Raju Ahamed, Miss Nushrat Jahan Lima, Tanjina Nasrin Eva, Md. Ashrafal Alam. 2024. Heavy metal intake by fishes of different river locations in bangladesh: a comparative statistical review. *Asian Journal of Fisheries and Aquatic Research*. 2024;26(6):43-67.
Available:<https://doi.org/10.9734/ajfar/2024/v26i6775>
 26. Shishir MKH, Sadia SI, Ahmed S, Aidid AR, Rana MM, Hasan MM, Alam MA. Transmission electron microscopic and X-ray diffraction based study of crystallographic bibliography demonstrated on silver, copper and titanium nanocrystals: State of the Art Statical Review. *Asian Journal of Applied Chemistry Research*. 2024;15(3):1-19.
 27. Khatun M, Kobir MM, Miah MAR, Sarkar AK, Alam MA. Technologies for remediation of heavy metals in environment and ecosystem: A critical overview of comparison study. *Asian Journal of Environment & Ecology*. 2024;23(4):61-80.
 28. Ahamed MS, Ali MS, Ahmed S, Sadia SI, Islam MR, Rahaman MA, Alam MA. Synthesis of silver nanomaterials capping by fruit-mediated extracts and antimicrobial activity: A critical review. *International Research Journal of Pure and Applied Chemistry*. 2024;25(1):45-60.
 29. Sarkar AK, Ahmed S, Sadia SI, Kobir MM, Tabassum S, Islam MR, Alam MA. (2024). Overview of the skeleton significance of toothpaste formulation, evaluation and historical perspectives: Insights from Bangladesh's toothpaste industry. *Journal of Materials Science Research and Reviews*. 2024;7(1):80-101.
 30. Kobir MM, Ali MS, Ahmed S, Sadia SI, Alam MA. Assessment of the physicochemical characteristic of wastewater in Kushtia and Jhenaidah Municipal Areas Bangladesh: A Study of DO, BOD, COD, TDS and MPI. *Asian Journal of Geological Research*. 2024;7(1): 21-30.
 31. Kobir MM, Tabassum S, Ahmed S, Sadia SI, Alam MA. Crystallographic benchmarking on diffraction pattern profiling of Polymorphs-TiO₂ by WPPF for Pigment and Acrylic Paint. *Archives of Current Research International*. 2024;24(1):62-70.
 32. Ali MS, Ahmed S, Islam MR, Ahamed MS, Rahaman MA, Khatun M, Alam MA. Diabetes mellitus control including fruits in diet: Exhaustive review and meta-analysis. *Asian Journal of Food Research and Nutrition*. 2024;3(1):43-59.
 33. Islam MR, Ahmed S, Sadia SI, Sarkar AK., Alam MA. Comprehensive review of phytochemical content and applications from *cestrum nocturnum*: A Comparative Analysis of Physicochemical Aspects. *Asian Journal of Research in Biochemistry*. 2023;13(4):43-58.
 34. Hasan MS, Jahan R, Alam MA, Khatun M, Al-Reza SM. Study on physicochemical properties of edible oils available in Bangladeshi local market. *Archives of Current Research International*. 2016;6(1):1-6.
 35. Moullick SP, Hossain MS, Al Mamun MZU., Jahan F, Ahmed MF, Sathee RA, Islam F. Characterization of waste fish bones (*Heteropneustes fossilis* and *Otolithoides pama*) for photocatalytic degradation of Congo red dye. *Results in Engineering*. 2023;20:101418.
 36. Rahman MM, Maniruzzaman M, Yeasmin MS, Gafur MA, Shaikh MAA, Alam MA, Quddus MS. Adsorptive abatement of Pb²⁺ and crystal violet using chitosan-modified coal nanocomposites: A down flow column study. *Groundwater for Sustainable Development*. 2023;23: 101028.

37. Andrade CJ, Andrade LM. Microalgae for bioremediation of textile wastewater. An overview. *MOJ Food Process Technol.* 2018;6(5):432-433.
38. Salam OEA, Reiad NA, ElShafei, MM. A study of the removal characteristics of heavy metals from wastewater by low-cost adsorbents. *Journal of Advanced Research.* 2011;2(4):297-303.
39. Sarayu K, Sandhya S. Current technologies for biological treatment of textile wastewater—a review. *Applied Biochemistry and Biotechnology.* 2012;167(3), pp.645-661.
40. April L. Ulery, Robert flynn & ramona parra appropriate preservation of dairy wastewater samples for environmental analysis, environmental monitoring and assessment volume. 2004;95:117–124.
41. Chen H, Pan SS. Bioremediation potential of Spirulina: Toxicity and biosorption studies of lead. *Journal of Zhejiang University. Science. B.* 2005;6(3): 171.
42. Haque NN, Alam MA, Baidya AS, Zenat EA, Rahman MZ, Roy CK, Munshi JL. Bioremedial capacity of indigenous hydrophytes and microalgae of Bangladesh: A comparative study on their potential in tannery effluent treatment. *Asian Journal of Environment & Ecology.* 2024;23(6);53-65.
43. Priya AK, Jalil AA, Vadivel S, Dutta K, Rajendran S, Fujii M, Soto-Moscoso M. Heavy metal remediation from wastewater using microalgae: Recent advances and future trends. *Chemosphere.* 2022;305: 135375.

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