

Sustainable Bioremediation of Textile Wastewater Using Bacterial Consortia and Its Reuse Potential for Irrigation

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A bacterial consortium was used to assess the potential of bioremediation for treating textile wastewater and evaluating the phytotoxicity effects of biotreated wastewater using tomato seed germination and seedling growth. The optimum conditions for bioremediation were achieved by varying the concentration of inoculum, temperature, incubation time and concentration of textile wastewater for maximum decolorization were 8 mL inoculum, 37 °C, 4 days incubation time, and 25 to 50 mL of the textile wastewater. The optimized treatment was implemented in a laboratory-scale bioreactor, resulting in substantial improvement in the measured wastewater-quality parameters. The COD removal reached approximately 86%, while TSS removal exceeded 80%. The untreated textile wastewater (TW) resulted in a decline in tomato seed germination and seedling development, whereas biotreated wastewater (Bt-W) improved growth performance. The percentage of germination in the control, TW, and Bt-W was 94%, 81%, and 90%, respectively. The seedling vigor index was highest in Bt-W (676.80), compared with the control (655.18) and TW (567.0). Under laboratory scale conditions, bacterial consortium-based biotreatment caused reduction of wastewater related phytotoxic effects and potential future development of reused wastewater from textile effluents. The approach supports the United Nations Sustainable Development Goals.

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INTRODUCTION

Biological treatment is one of the most recognised and widely applied methods for wastewater management in municipal and industrial practices. Activated sludge systems are one of the more widely used biological wastewater treatment technologies and rely on a diverse microbial community to degrade organic pollutants under aerobic conditions (Metcalf and Eddy 2014). Anaerobic digestion is also commonly used, such as sludge

stabilization and biogas production for resource recovery (Liu *et al.* 2020). Such processes are well-known and highlight the importance of microbial communities in real wastewater treatment systems, such as in organic matter degradation and resource recovery (Drewnowski *et al.* 2019).

However, industrial wastewater streams such as textile effluents remain challenging to treat due to their complex composition, which contains synthetic dyes, salts, heavy metals, and other persistent contaminants (Mohanty and Kumar 2021; Rai *et al.* 2024). Anthraquinone dyes can be degraded more effectively by microbial monocultures and developed consortia through the production of specific enzymes. While traditional biological treatment methods can be used to remove organic pollution, the removal of certain contaminants and associated toxicity may not be possible under specific wastewater conditions and operational parameters (Rai *et al.* 2024; Elnabi *et al.* 2025). Therefore, rather than replacing conventional biological treatment systems, targeted microbial approaches, including the application of selected bacterial consortia, are being investigated as complementary strategies to enhance treatment performance and reduce wastewater related toxicity (Elnabi *et al.*, 2025). The present study does not aim to replace established wastewater treatment technologies but evaluates whether a selected indigenous bacterial consortia can improve measured textile wastewater quality characteristics and reduce phytotoxic effects under laboratory-scale conditions. Indigenous bacterial consortia may provide additional treatment potential because microorganisms adapted to contaminated environments can possess synergistic metabolic capabilities that support the transformation of complex pollutants (Kumar *et al.* 2025).

Biological wastewater treatment systems are already based on the concept of microbial enrichment, with biomass retention and acclimation. In aerobic activated sludge systems, the continuous recirculation of microbial-rich sludge allows the development of diverse microbial communities which progressively adjust to the characteristics of the influent and operational conditions. Microbial community structure, stability, and functional adaptation to wastewater composition are closely linked to the effectiveness of activated sludge treatment (Chen *et al.* 2017; Dottorini *et al.* 2023). Likewise, anaerobic systems, such as sludge-bed reactors, depend on the presence of retained microbial biomass and structured sludge communities which enable the development of stable consortia and anaerobic degradation pathways, including syntrophic metabolism and methanogenesis (Duan *et al.* 2021; Wu *et al.* 2021). These proven treatment methods have shown that acclimation of the microorganisms and selection of the microbial community are key concepts in the successful operation of wastewater treatment processes.

Intentional bioaugmentation with selected microorganisms differs from the natural development of microbial communities in conventional reactors. Purposeful inoculation involves the intentional addition of microorganisms that possess a distinct functional capability that can improve treatment of specific contaminants or speed the adaptation process in the face of difficult conditions, whereas activated sludge and anaerobic systems rely on the ability of the microbial community to adapt over time by maintaining biomass for a extended solids retention periods.

Nevertheless, microbial community composition remains dynamic and is influenced by influent properties, ecological interactions, and reactor operating conditions (Dottorini *et al.* 2023; Duan *et al.* 2021). Thus, the potential value of the selected bacterial consortia is not to replace the existing biological treatment systems, but to act as a complement to the existing microbial process for specific functions, especially in complex industrial wastewater streams such as textile effluents.

Phytotoxicity assays are frequently carried out with tomato seeds as bioindicators because of their sensitivity (Baruah *et al.* 2019). The tomato plant, *Solanum lycopersicum*, is a widely used bioindicator plant in phytotoxicity testing because of its low tolerance to environmental contaminants and the fast rate of germination. Seeds can be used as a good reproducible indicator of toxicity, as small fluctuations in concentrations of pollutants can lead to large variations in germination, root elongation and seedling growth (Gautam *et al.* 2015). Tomatoes also have agricultural significance, and the results of the wastewater toxicity or remediation strategies can be directly applied to crop production and food safety. Moreover, it has been seen that the use of tomato seeds to test treated effluents can indicate better germination and vigour of the seedlings, which is a good indicator to measure the efficiency of bioremediation (Baruah *et al.* 2019). They are easy to handle in laboratory conditions, have a fast growth rate, and have well characterised physiology making them suitable for controlled experiments using bioreactors and pollutant degradation studies (Kumar *et al.* 2017). Untreated textile effluents can cause a reduction in the germination rate and growth of seedlings because of the presence of dyes and toxic substances that can inhibit seed germination and seedling growth (Rehman *et al.* 2008; Khan and Malik 2018). However, the effluents generated from the biotreated wastes usually show low toxicity, and at times improve the germination rate and seedling vigour, owing to the good degradation and reduction of hazardous substances by the biological activity in the treated effluents (Alrowais *et al.* 2023).

While naturally occurring, microbial communities are used in conventional wastewater treatment systems, the addition of selected microorganisms can offer further advantages for certain wastewater treatment problems. Bioaugmentation is the intentional addition of selected microbial strains or mixed cultures to increase the degradation of specific contaminants or to increase the effectiveness of the treatment (Herrero and Stuckey 2015). The bacterial consortium was not used as an alternative to existing biological treatment technologies, but rather as a targeted approach, under optimized laboratory conditions, in the present study. Environmental conditions such as inoculum concentration, temperature, treatment time, and wastewater concentration were optimized to facilitate the activity of the consortium, as environmental factors can have a significant impact on the survival, adaptation, and efficiency of the consortium in the removal of pollutants during the bioaugmentation process (Chettri *et al.* 2024). Thus, the aim of this study was to investigate whether a selected indigenous bacterial consortium can improve the performance of the textile wastewater treatment beyond the general microbial activities and reduce the phytotoxicity of the wastewater.

The present study aimed to (i) optimize selected bacterial consortia isolated from textile wastewater; (ii) apply bioremediation using designed bioreactor system with textile effluents; (iii) evaluate the changes in the key physicochemical parameters prior to and after bioremediation; (iv) measure the percentage of reduction of metals (Ni, Cu, Cr, Zn, Co, Mn, Pb, Cd) in textile wastewater; and (v) conduct a comparative study of seed germination and growth of seeds in control (tap water) conditions, untreated (TW) and biotreated wastewater (Bt-W). The above goals were designed to gain insights into the potential sustainable use of microbial consortia for environmentally friendly and potentially scalable treatment of textile effluents (Singh *et al.* 2021). The objective was to evaluate changes in measured wastewater-quality parameters and contaminant concentrations following treatment and to assess the phytotoxic response of untreated and biotreated wastewater using tomato seed germination and seedling growth.

EXPERIMENTAL

Materials and Methods

Sample collection

Textile wastewater samples were collected in pre-cleaned and sterilized containers from seven distinct textile industrial sites located along the 12 km Lahore-Sheikhupura-Faisalabad Rd, Kot Abdul Malik in Pakistan. For the development of bacterial consortia BC-23, the strains with the highest decolorization percentages were selected from 67 isolates. The consortium comprised one indigenous isolate of *Bacillus subtilis* (JF70263.1) and three strains of *Bacillus cereus* with accession numbers of MG563677.1, OP572138.1, and LC052668.1, respectively. This was used as inoculum for the bioremediation of textile wastewater.

Optimization of Bioremediation Parameters

The optimization experiments were performed sequentially rather than independently and included inoculum concentration, temperature, time duration, and concentration of TW. The reason for optimization trials was to determine the most appropriate conditions meant for active bioremediation, which was assessed by decolorization efficiency before scaling up the activity in the bioreactor (Jimoh *et al.* 2025).

Bioreactor Design

A laboratory-scale batch bioreactor made of stainless steel, with a total capacity of 25 L, was used for the experiment. Approximately 15.5 L was maintained as the working volume, while the remaining capacity served as headspace above the liquid surface during batch operation. The cylindrical bioreactor was equipped with an electrically driven mechanical stirrer to ensure continuous mixing and promote uniform distribution of reactor contents throughout the wastewater matrix (Zaburko *et al.* 2021). The lower section of the vessel allowed settlement of biomass and particulate material, while a sampling outlet located above this region was used for collection of biotreated wastewater (Bt-W). Mechanical agitation was maintained continuously throughout the 8-day treatment period using an electrically driven stirrer to ensure adequate mixing of the reactor contents with the agitation rate estimated to be approximately 100 to 200 rpm. Temperature was maintained between 37 and 39 °C using an external heat source, which corresponds to a suitable range for mesophilic microbial activity (Meena *et al.* 2022). The system was operated as a non-aerated batch treatment under mixed conditions rather than as a strictly anaerobic process. Although the reactor contained a lower biomass settlement region, it was not operated as an upflow anaerobic sludge blanket (UASB) reactor because no continuous influent or effluent flow was maintained. The reactor was used to evaluate short-term bioremediation performance under controlled laboratory conditions. The pH was monitored using a pH probe and maintained within the range of 7.5 to 8.0 throughout the treatment period.

Inoculum Preparation and Bioreactor Operation

Initially, each strain was cultured on separate NA (nutrient agar) plates and incubated for 24 h at 37 °C to attain active colonies. The prepared nutrient broth media was distributed into twelve 500 mL conical flasks (three flasks per bacterial isolate). Each flask was inoculated with a loopful of specific bacterial strain and incubated for 6

h at 37 °C under shaking at 130 to 150 rpm. Broth cultures were mixed thoroughly to form a homogeneous mixture of bacterial consortium and were subsequently incubated at 37 °C for 24 h to promote microbial activity and consortium stability. Next, the developed consortium of 3.5 L and was added into the 12 L of TW in bioreactor. The system was operated under controlled conditions to facilitate bioremediation. This approach was consistent with previous findings (Desai *et al.* 2009).

Each laboratory scale bioreactor experiment was conducted as one independent batch treatment. The biotreated wastewater obtained from this single eight-day batch was subsequently used for physicochemical characterization, heavy-metal analysis, and phytotoxicity assessment. Accordingly, repeated measurements performed on aliquots of this treated wastewater, where applicable, represent analytical or technical replicates rather than independent biological replicates of the bioreactor treatment.

Bacterial Inactivation Under Acetic Acid Stress

Bacterial inactivation was done by subjecting the Bt-W to acetic acid stress which was added at a concentration of 0.05 mL/L, and the pH of the medium was noted. The biotreated effluent bearing acetic acid was consequently cultured for 24 h on nutrient agar plates to evaluate the colony-forming ability (CFA) of the bacterial consortium (Gravemann *et al.* 2018), the plates were examined for bacterial growth to determine the effectiveness of acetic acid treatment in reducing or eradicating viable bacterial colonies (Gomaa 2012; Hua *et al.* 2024).

Preparation TW and Bt-W for Seed Germination

Three types of water were used in the germination and seedling growth experiment: tap water, untreated textile wastewater, and biotreated textile wastewater. Tap water was used as the control treatment, while untreated textile wastewater (TW) and biotreated wastewater (Bt-W) were used as the experimental treatments. Before application, both TW and Bt-W were diluted by preparing a 70:30 mixture, consisting of 70% wastewater and 30% clean water. The use of different wastewater concentrations is commonly applied in textile wastewater phytotoxicity studies to evaluate plant responses under controlled exposure levels and to compare the effects on seed germination, radicle, and shoot growth, and biomass production (Das *et al.* 2019). Seed germination and seedling growth responses were later assessed using germination and phytotoxicity-related indices, following the general approach of seed-based phytotoxicity bioassays and germination index assessment (Kong *et al.* 2023). The seed germination experiment was performed using three replicates for each treatment group (tap water, untreated textile wastewater, and biotreated wastewater).

Experimental Design for Germination

The tomato seeds were first washed with dH₂O to remove dust and dirt from the surface of the seed and then dipped in 2% sodium hypochlorite (NaOCl) solution for 30 minutes (George *et al.* 2008). This treatment was found to be effective in killing fungi, bacteria, and other contaminants on the seed coat (Cassells 2012). The seeds were then washed with sterile dH₂O to remove all traces of NaOCl solution to ensure that no chemicals are present to affect seed germination (Bhojwani and Razdan 1986). Sterilized seeds (100 each) were placed in containers lined with filter paper and moistened periodically with tap water, textile wastewater, or biotreated water as needed to keep the paper moist throughout the germination (Bharti *et al.* 2018). The seedlings of different

days were used to measure the radicle and plumule length to check the performance of the treatments. At the end of the experiment, germination parameters including plumule and radicle length were recorded and compared (Zouagri *et al.* 2020). Each treatment was conducted in triplicate ($n = 3$) to account for biological variation among seedlings. Radicle and plumule measurements were expressed as mean $\pm$ standard deviation (SD) based on replicate observations.

Physicochemical Characterization of TW and Bt-W

Characterization of the TW and Bt-W for various parameters such as temperature, color, pH, electric conductivity (EC), chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solids (TDS), and total suspended solids (TSS) was conducted before and after the bioremediation by bacterial consortium (Bashir *et al.* 2023). Physicochemical parameters were measured using samples obtained before and after the single bioreactor treatment. Each analysis was performed in triplicate ($n = 3$) and represents analytical replicates of the same wastewater sample rather than independent biological replicates of the bioreactor treatment.

Statistical Analysis

Statistical analysis was performed using SPSS, version 24. Descriptive statistics were used to summarize the experimental observations. Radicle and plumule lengths were expressed as mean $\pm$ standard deviation (SD) based on three replicate observations ($n = 3$), as reported in Tables 2 and 3. Other treatment outcomes, including physicochemical parameters, heavy-metal concentrations, percentage reductions, germination counts, and derived germination indices, were evaluated descriptively using the observed values and calculated treatment-response measures. Because the laboratory-scale bioreactor treatment was conducted as a single independent batch, inferential statistical testing was not applied to comparisons between untreated and biotreated wastewater. Therefore, no significance threshold was applied to these single-batch comparisons, and statistical significance was not inferred from the observed differences.

RESULTS AND DISCUSSION

Optimization of Bioremediation Conditions for TW Decolorization

Bacterial consortia were subjected to optimization by the concentration of inoculum, temperature, incubation period, and TW concentration to increase bioremediation efficiency (Fig. 1). The decolorization efficiency increased gradually up to an inoculum concentration of 8 mL, at which point the highest decolorization response was observed. It indicated a balance of a suitable level of microbial biomass for effective activity, and the accuracy of the findings was further confirmed by the increasing trend followed by a slight decline, which is consistent with the findings of El-Bendary *et al.* (2023). Temperature also strongly affected microbial activity. Decolorization efficiency increased from 30 °C to 37 °C, reaching a maximum of 49% at 37 °C, before decreasing to 43% at 60 °C, which is quite consistent with studies done earlier (Gomez *et al.* 2020). The observed mesophilic optimum supports microbial physiology, whereby the enzyme systems operate optimally at moderate temperatures. At higher temperatures, the enzymatic systems have been found to become partially denatured and inactive (Paissoni *et al.* 2024). This classical trend further supports reproducibility of the results. Decolorization was

highly influenced by the incubation period. Efficiency rose gradually between day 1 and day 4, then sharply dropped on day 5 and leveled off at 48% (Ikram *et al.* 2022a). This trend may reflect microbial growth dynamics, with the highest decolorization response occurring during the earlier treatment period and declining at longer incubation times, potentially because of nutrient limitation and the accumulation of metabolic products (Fig. 1). Closeness to known microbial kinetics increases the reliability of the findings (Ikram *et al.* 2022b). The quantity of wastewater was negatively correlated with the degradation efficiency. Optimal decolorization of 18% was achieved at 25 mL, with a progressive decline in decolorization as the volume increased to 150 mL with 6% decolorization. This is consistent with pollutant overload, oxygen limitation, and recalcitrance effects documented for textile effluents (Zafar *et al.* 2022). This observation supports the reliability of the results and highlights important practical implications, such as the need for pretreatment or dilution to enhance treatment efficiency. Overall, the systematic variation of parameters and the consistency of the observed trends reinforce the validity of the findings. Finally, the most suitable conditions were 8 mL inoculum, 37 °C, 4 days of incubation, and a range of 25 to 50 mL wastewater concentration to attain maximum decolorization in an experimental flask.

Monitoring Bioreactor and Bacterial Growth

The bioreactor system was continuously monitored to regulate microbial activity during the biotreatment process. For bioreactor operation, 3.5 L of inoculum was prepared with 875 mL per strain distributed in 12 conical flasks (three flasks per bacterial isolate) of 500 mL. The system contained a total liquid volume of 15.5 L, including 12 L of TW and 3.5 L of inoculum.

The pH was maintained between 7.5 and 8, and continuous stirring was carried out for a period of 8 days (Fig. 2). Although maximum decolorization was achieved after approximately four days during flask-scale optimization (Fig. 1C), the laboratory-scale bioreactor experiment was conducted for eight days. The optimization experiment was performed using a lower wastewater volume and a different experimental configuration compared with the subsequent bioreactor treatment. The larger working volume and bioreactor configuration may affect microbial contact, mixing conditions, and biotreatment kinetics during scale-up (Garcia-Ochoa and Gomez 2009).

Acetic acid is used as a control agent against undesirable bacterial growth, and it is in line with the studies indicating that organic acids would have an inhibitory effect on the bacterial metabolism and its capacity (Park *et al.* 2021). The experimental results showed that growth of the bacteria was greatly inhibited in the presence of acetic acid. All bacterial isolates in the consortium, however, were still viable under normal operating conditions, indicating their suitability and stability for bioremediation applications (Sun *et al.* 2021). When the isolates were treated with acetic acid, however, the acids were not found to be able to withstand the harsh environment, which shows the inhibitory effect of acetic acid on microbial growth (Huang *et al.* 2011).

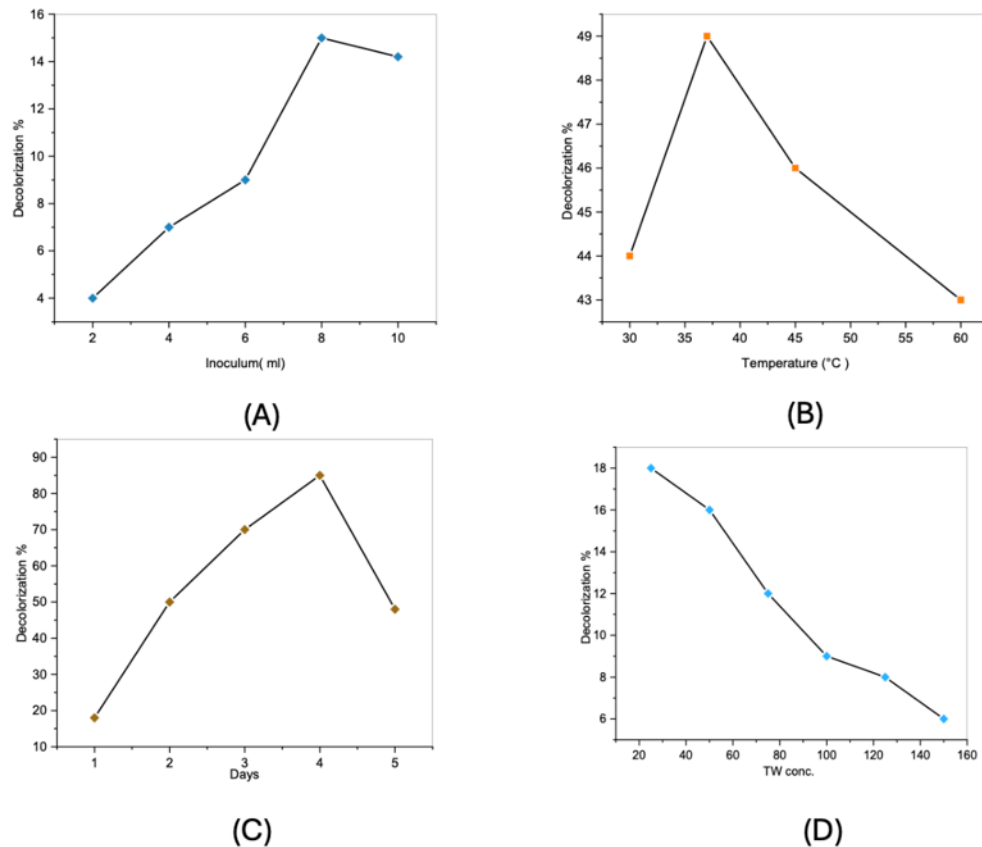


Fig. 1. Sequential optimization of treatment parameters affecting decolorization efficiency, including inoculum concentration, temperature, incubation period, and wastewater concentration: (A) Effect of inoculum concentration; (B) Effect of temperature; (C) Effect of incubation period; and (D) Effect of TW concentration

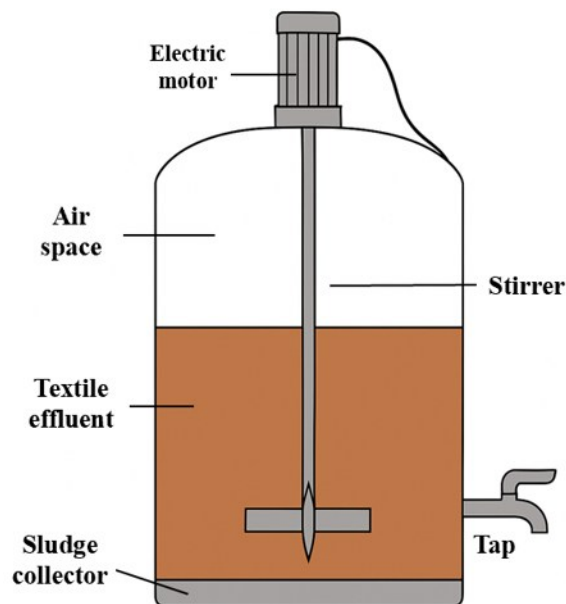


Fig. 2. Schematic diagram of the laboratory-scale bioreactor

Physicochemical Characterization of Untreated and Biotreated Water

Rate of fermentation

The physicochemical parameters measured before and after treatment were used to evaluate the performance of the bioremediation process. A marked decrease was observed in EC, TDS, TSS, BOD₅, and COD following biotreatment, indicating an overall reduction in the measured pollution indicators (Fig. 3). The reduction in the level of COD was around 85 to 87%, indicating substantial reduction in the oxidizable load of the effluent (Sun *et al.* 2020). However, a reduction in COD does not always indicate biodegradation or complete mineralization, as it included parts that may not be biodegradable. In addition to biological transformation, a reduction of COD can also occur due to adsorption to biomass, precipitation, sedimentation, or biomass-associated removal (Wei *et al.* 2019). Therefore, the relative contribution of these processes and complete mineralization to CO₂ and water cannot be established from COD measurements alone (Lacalamita *et al.* 2024; Fertil *et al.* 2025). In the same way, TSS removal exceeded 80%. This indicates that the suspended solids were reduced effectively. Conversely, EC and TDS showed moderate reductions indicating the persistence of dissolved inorganic salts (Fig. 4).

Overall, these findings suggested the effectiveness of microbial consortium in enhancing the quality of wastewater. The textile wastewater quality analysis both before and after biotreatment is effectively demonstrated through Figs. 3 to 5. The untreated textile wastewater (TW) exhibited elevated EC, TDS, TSS, BOD₅, and COD, indicating a substantial pollution load (Fig. 3), which is consistent with the characteristics commonly reported for textile-industry effluents (Yaseen and Scholz 2019; Wang *et al.* 2022). These parameters decreased considerably following biological treatment, together with observed reductions in color and odor, indicating an overall improvement in the measured wastewater-quality characteristics (Holkar *et al.* 2016; Ceretta *et al.* 2020). However, the analyses presented in the current manuscript do not establish the specific mechanisms responsible for these changes.

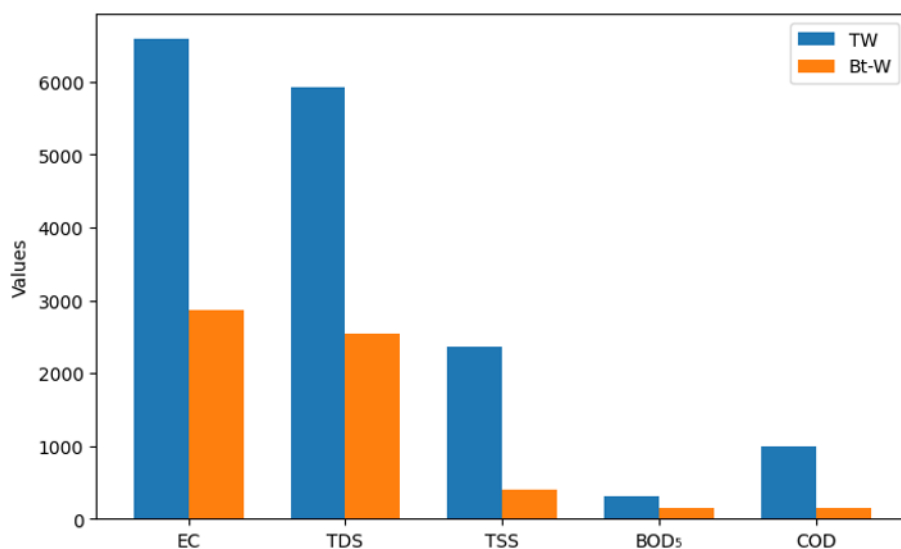


Fig. 3. Comparative physicochemical characteristics of untreated textile wastewater (TW) and biotreated textile wastewater (Bt-W)

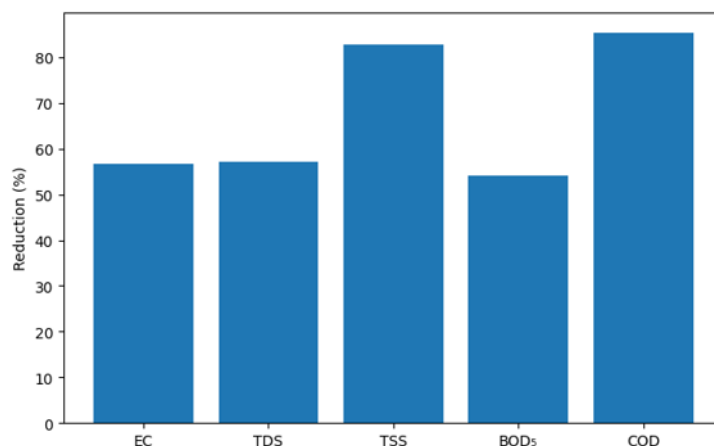


Fig. 4. Percentage reduction of selected wastewater quality parameters following biotreatment. The figure provides a comparative assessment of treatment efficiency, while the corresponding measured values before and after treatment are presented in Fig. 3.

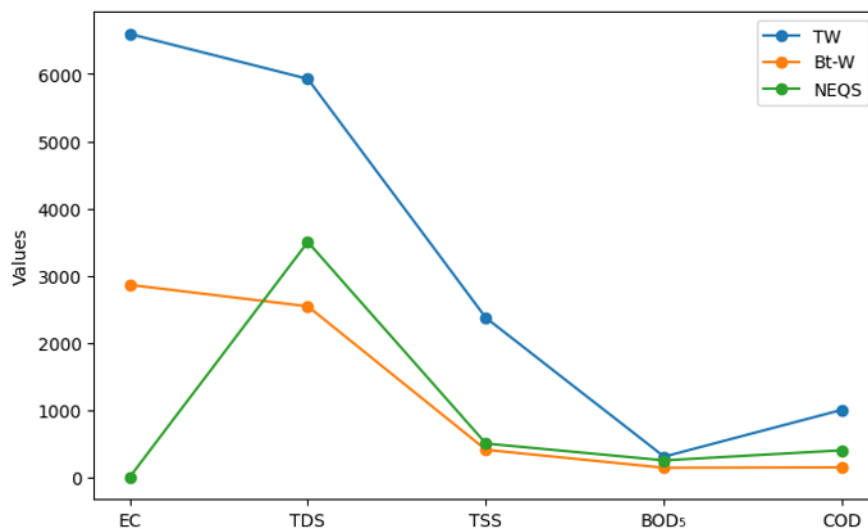


Fig. 5. Comparison of wastewater quality parameters with NEQS standards before and after biotreatment

Comparative Assessment of Textile Water

Metal concentrations in untreated (TW) and biotreated wastewater (Bt-W) samples were determined using inductively coupled plasma optical emission spectrometry (ICP-OES). Each sample was analysed in duplicate ($n = 2$), and the results were expressed as mean $\pm$ standard deviation (SD) where applicable. Since the bioreactor treatment was conducted as a single independent batch, these replicate measurements represent analytical variation of the same wastewater samples rather than independent biological replicates of the treatment process. Comparison with the prescribed NEQS limits suggests the efficacy of bioremediation in dropping metal contamination. Untreated TW showed higher Cu, Co, Mn, and Cd levels, supporting the presence of high concentrations of heavy metals commonly found in textile effluents. The bacterial consortium treatment notably reduced the concentration of most metals, showing effective bioremediation potential of the consortium in textile wastewater management.

The concentrations of some metals in the aqueous phase decreased following the reported biotreatment, but the extent of the decrease varied from one metal to another (Fig. 6). Cu showed one of the strongest treatment responses, while Ni, Zn, Mn, Cd, and Co also decreased to varying degrees. This trend of removal of elements is consistent with previous studies showing that microbial consortia do not remove all metals at the same rate. For instance Sannasi *et al.* (2010) reported variable removal of Cu, Pb, Cd, and Ni using an environmental bacterial consortium, while Qurbani *et al.* (2025) reported different removal efficiencies for Cu, Zn and Ni using a mixture of *Pseudomonas putida* and *Pasteurella aerogenes*. These variations may reflect differences in metal speciation, initial concentration, pH, competing ions, biomass concentration, microbial composition, contact time, and availability of metal binding sites (Igiri *et al.* 2018; Razzak *et al.* 2022; Qattan 2025). The observed decreases represent reductions in measured aqueous metal concentrations rather than evidence of a specific removal mechanism, but rather a decrease in the measured aqueous metal concentration. Unlike organic contaminants, which may undergo biological degradation and transformation, metals cannot be biodegraded; however, microorganisms may immobilize, accumulate, precipitate, transform or redistribute metals into different dissolved, particulate and biomass-associated fractions (Igiri *et al.* 2018; Medfu *et al.* 2020). The possible processes that can contribute to the observed reductions are passive biosorption onto the functional groups of cell walls and extracellular polymeric substances, biomass-associated accumulation, biologically influenced precipitation, redox-mediated changes in metal speciation, and sedimentation of metal-bearing biomass (Razzak *et al.* 2022; Qattan 2025). The present results, however, do not allow conclusions regarding the relative contribution of these processes because metal speciation, biomass-bound metals, sludge composition and mass balances were not investigated.

The residual Ni, Mn, Cd, and Co concentrations after the treatment showed that there is a potential to further optimize treatment performance where concentrations are still above the applicable regulatory limits, whereas the concentration of Zn was not above the applicable regulatory limit. The incomplete or varying removal of metals may be due to competition for binding sites, difference in metal speciation, and microbial responses in the complex wastewater matrix (Igiri *et al.* 2018; Qasem *et al.* 2021). The impact of pH, biomass concentration, treatment time, mixing conditions, and other operational parameters should be studied in the future to understand their role on the efficiency of metal removal. Complementary processes, such as adsorption, chemical precipitation, ion exchange, membrane separation, or electrochemical treatment, may also be considered where lower residual metal concentrations are required (Barakat 2011; Qasem *et al.* 2021). Cr and Pb were not detected in either biotreated or untreated wastewater. The lack of detection does not necessarily mean that they are not present, as concentrations below the analytical sensitivity of the method cannot be ruled out. Concentrations and relative abundances of metals in textile effluents can also be quite different depending on the type of fibers being used, dyes/pigments being used, auxiliary chemicals and the manufacturing process. (Yaseen and Scholz 2019; Velusamy *et al.* 2021). Therefore, the absence of detection of Cr and Pb in the biotreated wastewater should not be attributed to treatment because these metals were also not detected in untreated TW; their non-detection may instead reflect low initial concentrations, production-specific inputs, or analytical sensitivity.

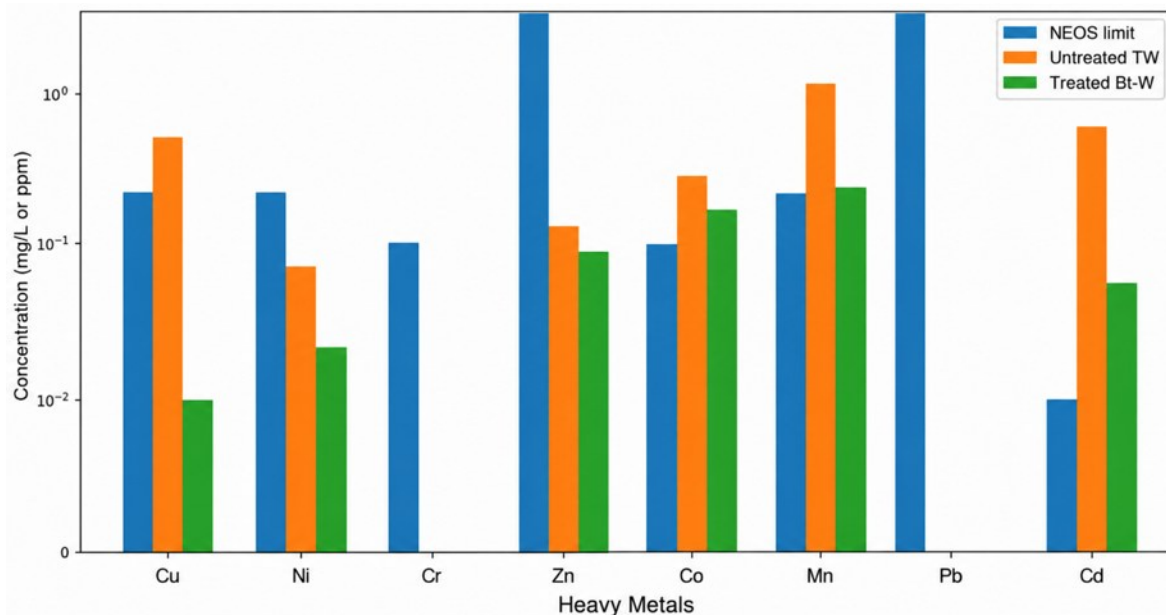


Fig. 6. Comparison of heavy metal concentrations in TW and Bt-W with NEQS permissible limits

Germination and Growth Performance

Radicle development varied among treatments and over time (Figs. 7 to 9). Bt-W showed greater early radicle growth, but this pattern was not sustained. TW produced relatively long radicles at some time points but did not result in better plumule development or overall germination performance (Tables 2 and 3; Fig. 9). This comparison indicates that root elongation alone is not sufficient to represent overall seedling performance. Root and shoot responses to abiotic stress can differ, as plants may alter root-system architecture and biomass distribution under resource limitations and other environmental constraints (Comas *et al.* 2013; Karlova *et al.* 2021; Shoaib *et al.* 2022). For example, maize primary roots have been shown to continue elongating at water potentials that inhibit shoot growth (Sharp *et al.* 1988). The comparatively greater radicle elongation observed under TW may therefore reflect differential root and shoot sensitivity or a stress-associated allocation response rather than a general stimulation of seedling growth (Sharp *et al.* 2004; Karlova *et al.* 2021). The treatments were more clearly differentiated by plumule development. Bt-W showed greater plumule growth, reaching approximately 5.0 to 6.0 cm on days 6 to 7 and remaining slightly above the control on day 18. TW, on the other hand, produced shorter plumules during the later growth period, indicating a contrasting root and shoot growth response (Table 3; Fig. 9).

Similar studies indicate that the effects of textile wastewater on seedling development depend on effluent concentration, composition, and plant species. High concentrations of textile effluent have been associated with reductions in germination, root and shoot development, biomass, and physiological performance, whereas lower concentrations may sometimes stimulate individual growth parameters (Khan *et al.* 2011; Rahman *et al.* 2018; Kurmi *et al.* 2023). These concentration-dependent responses may help explain why TW produced comparatively high radicle lengths at individual observations while still restricting plumule development and overall seedling performance. Radicle elongation, plumule elongation, and germination are complementary phytotoxicity endpoints and may differ in their sensitivity to wastewater constituents (Priac *et al.* 2017; Khelil *et al.* 2022). Although root elongation is frequently used as a sensitive endpoint

because roots are in direct contact with the test solution, interpretation based on root length alone may overlook adverse effects on shoot development and overall seedling vigour (Priac *et al.* 2017). The relatively high TW radicle values should therefore be interpreted together with plumule length, germination percentage, and seedling-vigour indices (Tables 4 and 5).

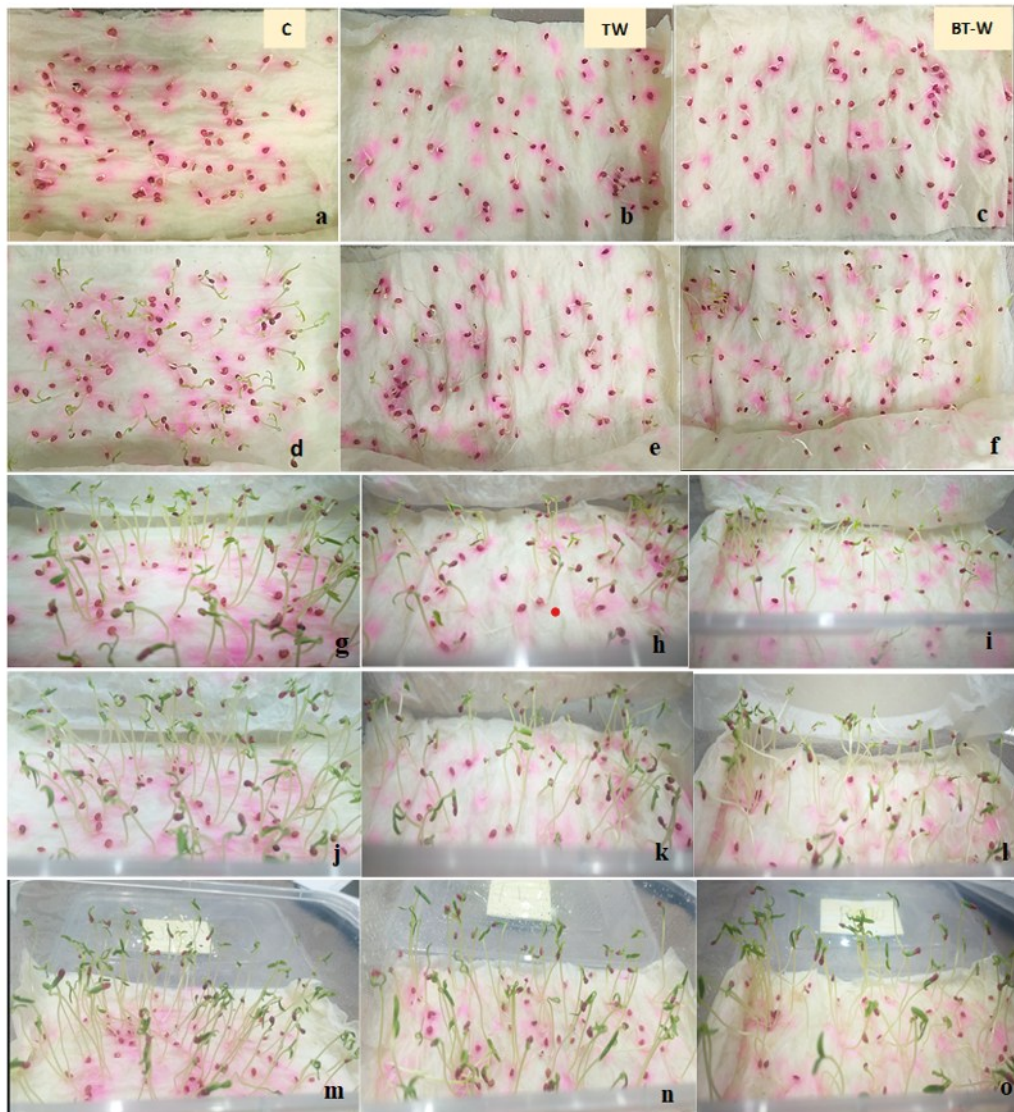


Fig. 7. Germination progression of tomato seeds under different water treatments. Panels (a, b, c) represent seeds on the 3rd day; (d, e, f) on the 4th day; (g, h, i) on the 5th day; (j, k, l) on the 6th day; and (m, n, o) on the 7th day. Seeds were grown using tap water, textile wastewater, and biotreated water, highlighting variations in germination performance over time.

The more balanced radicle and plumule development observed under Bt-W is consistent with reduced phytotoxic effects following treatment. Untreated wastewater was found to have phytotoxic effects on seed germination and seedling growth, while bacterial treatment was found to decrease the observed phytotoxic effects (Fendri *et al.* 2013). Ravindran *et al.* (2016) also found that wastewater treatment resulted in better germination and early growth responses.

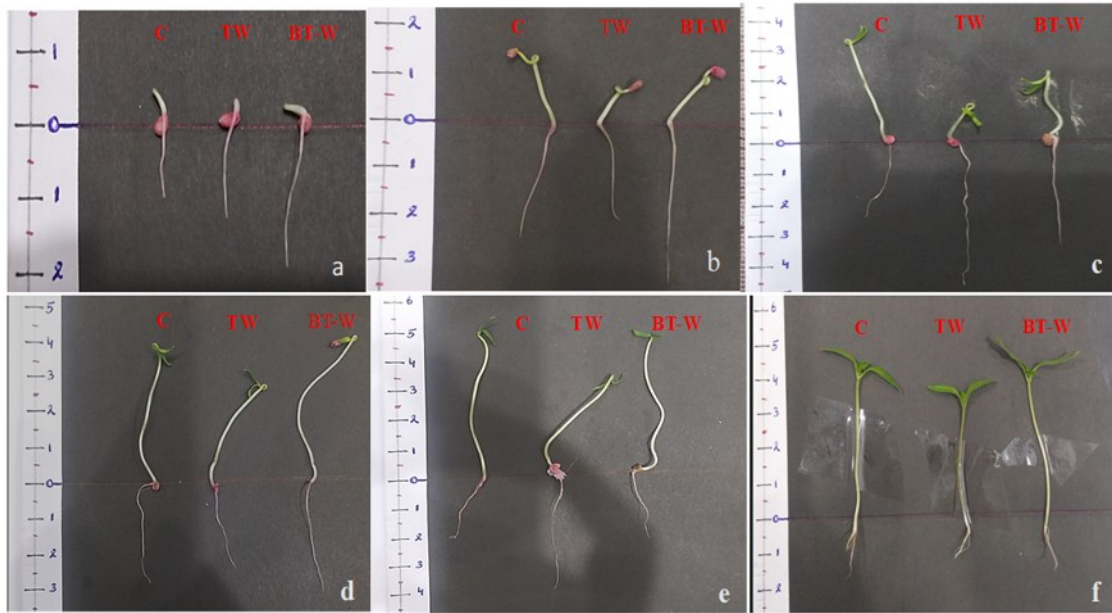


Fig. 8. Germination progression and seedling growth of radicle and plumule across 3rd, 4th, 5th, 6th, 7th, and 18th day

These studies were performed with domestic wastewater instead of textile wastewater, but they corroborate the general finding that biological treatment can decrease the phytotoxicity of wastewater. This interpretation is supported by Akshaya Vidhya *et al.* (2022), who found that the germination rate and root and shoot growth of *Vigna radiata* were better in the biologically treated synthetic azo-dye wastewater than in the untreated wastewater. These findings further emphasize that radicle, plumule, and germination responses should be interpreted collectively because individual seedling endpoints and plant species may differ in their sensitivity to wastewater constituents (Teixeira *et al.* 2024).

Following these root and shoot responses, germination performance also showed a clear descriptive pattern among the treatments. The control maintained the highest final germination, with 94 of 100 seeds germinating by day 18, followed closely by Bt-W with 90 seeds, whereas TW reached 81 seeds (Table 1).

Table 1. Germination Count of Tomato Seeds Grown in Tap Water (C), Textile Wastewater (TW), and Biotreated Textile Water (Bt-W)

	No. of Seeds						
	Total	3 rd	4 th	5 th	6 th	7 th	18 th
C	100	85	87	89	90	91	94
TW	100	70	75	77	78	78	81
BT-W	100	82	84	85	86	88	90

Table 2. Radicle Length (cm) under Different Treatments Over Time (mean $\pm$ SD, n = 3 replicate observations)

	Radicle (cm)					
	3 rd	4 th	5 th	6 th	7 th	18 th
C	1.0 $\pm$ 0.1	2.5 $\pm$ 0.1	3.0 $\pm$ 0.1	3.7 $\pm$ 0.1	3.1 $\pm$ 0.1	3.3 $\pm$ 0.1
TW	1.4 $\pm$ 0.1	1.9 $\pm$ 0.1	5.0 $\pm$ 0.1	3.1 $\pm$ 0.1	4.4 $\pm$ 0.1	4.7 $\pm$ 0.1
BT-W	2.1 $\pm$ 0.1	3.5 $\pm$ 0.1	3.4 $\pm$ 0.1	3.5 $\pm$ 0.1	2.8 $\pm$ 0.1	3.2 $\pm$ 0.1

Table 3. Plumule Length (cm) under Different Treatments Over Time (mean $\pm$ SD, n = 3 replicate observations)

	Plumule (cm)					
	3 rd	4 th	5 th	6 th	7 th	18 th
C	0.5 $\pm$ 0.1	2.2 $\pm$ 0.1	4.0 $\pm$ 0.1	4.0 $\pm$ 0.1	5.7 $\pm$ 0.1	4.3 $\pm$ 0.1
TW	0.2 $\pm$ 0.1	1.5 $\pm$ 0.1	1.7 $\pm$ 0.1	3.5 $\pm$ 0.1	4.2 $\pm$ 0.1	3.5 $\pm$ 0.1
BT-W	0.5 $\pm$ 0.1	2.0 $\pm$ 0.1	2.2 $\pm$ 0.1	5.0 $\pm$ 0.1	6.0 $\pm$ 0.1	4.5 $\pm$ 0.1

The relatively close germination response of Bt-W to the control, together with its greater plumule development, suggests that biotreatment reduced the inhibitory effects associated with untreated wastewater. Comparable studies have reported improved germination and reduced phytotoxicity following biological treatment of textile- or dye-containing wastewater (Sonu *et al.* 2021; Selim *et al.* 2021; Akshaya Vidhya *et al.* 2022). When germination counts are considered together with the seedling-growth responses, Bt-W showed a more balanced overall response than TW (Tables 1–5; Fig. 9).

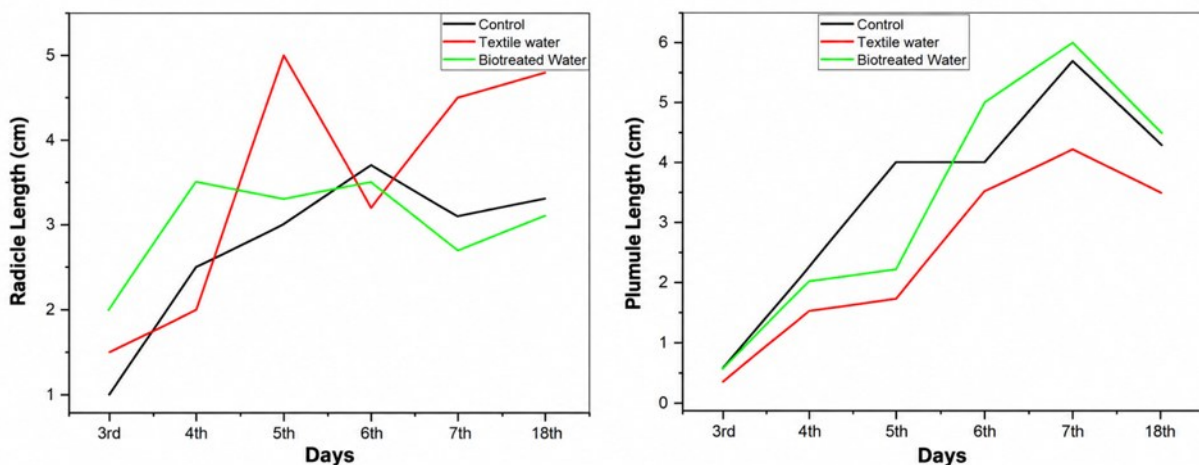
**Fig. 9.** Effect of TW and Bt-W on seedling development over time: (left) radicle length and (right) plumule length compared to control

Table 4. Germination and Seedling Growth Indices, Formulas, Explanations, and References Used To Evaluate Seed Germination Performance, Seedling Vigor, Stress Tolerance, and Phytotoxicity Under Control and Treatment Conditions

Germination Terms	Formula	Explanation of Formula	Notes with Reference
Germination Percentage (GP)	$GP\% = \frac{\text{Number of seeds germinated}}{\text{Total no. of seeds}} \times 100$	Measures the proportion of seeds that germinated	Higher GP values indicate greater rate of germination in the seed population (Scott <i>et al.</i> 1984).
Mean Germination Time (MGT)	$MGT = \frac{\sum(G \times T)}{\sum G}$	G= No. of seeds germinated on a specific day T=time (days) since the start of germination $\sum G$= Total no. of seeds germinated $\sum(G \times T)$ = It is the summation of seeds germinated at each time point multiplied by time	Indicates the average time it takes a seed to germinate. Lower MGT means faster germination (Orchard 1977).
Coefficient of Velocity of Germination (CVG)	$CVG = \frac{100 \times \sum G}{\sum(G \times T)}$		The CVG formula clearly emphasizes the speed and spread of germination. The highest possible CVG value is 100, which indicates that all seeds germinate on the first day. Higher CVG indicates faster germination (Kotowski 1926).
Germination Rate Index (GRI)	$GRI = \frac{G_1}{T_1} + \frac{G_2}{T_2} + \dots + \frac{G_n}{T_n}$	$G_1, G_2, \dots, G_n$ = Number of seeds germinated on each day; $T_1, T_2, \dots, T_n$ = corresponding days.	GRI measures the speed of germination; its high value shows higher and faster germination (Maguire 1962).
Germination Index (GI)	$GI = \frac{RSG \times RRG}{100}$	$RSG = \frac{\text{No. of seeds germinated in sample}}{\text{No. of seeds in control}} \times 100$ $RRG = \frac{\text{Mean radicle length of sample}}{\text{Mean radicle length of control}} \times 100$	This index integrates both germination response and radicle growth to provide an overall assessment of wastewater-associated phytotoxicity (Zucconi <i>et al.</i> 1985)

Germination Terms	Formula	Explanation of Formula	Notes with Reference
Stress Tolerance Indices (STIs)	Includes RLSTI, SLSTI, RFSTI, SFSTI, RDSTI, and SDSTI.	These indices compare seedling growth under stress with growth under control conditions.	It monitors high-yielding and stress-tolerating ability in crop plants (Rashid <i>et al.</i> 2021; Wang <i>et al.</i> 2025).
Radicle Length Stress Tolerance Index (RLSTI)	$(RLSTI) = \frac{\text{radicle length of stress plant}}{\text{radicle length of control plant}} \times 100$	Compares radicle length under stress and control conditions.	A higher percentage of RLST1 indicates the efficiency of biotreated water in radicle development.
Shoot Length Stress Tolerance Index (SLSTI)	$(SLSTI) = \frac{\text{shoot length of stress plant}}{\text{shoot length of control plant}} \times 100$	Compares shoot length under stress and control conditions.	A high percentage of SLST1 indicates the efficiency of biotreated water in shoot development.
Seedling Vigor Index (SVI)	$SVI = (rL + sL) \times GP$	rL=mean radicle length; sL= mean shoot length GP= germination percentage	A higher SVI indicates stronger and more vigorous seedlings. Zhao <i>et al.</i> (2021)
Phytotoxicity Index (PI)	$PI = 1 - \frac{SRL}{CRL}$	SRL= Radicle length of sample; CRL= Radicle length of control	Positive PI values indicate a toxic effect on seeds, whereas negative values show better growth than the control (Rashid <i>et al.</i> 2021).

Table 5. Comparative Germination Parameters and Stress-Tolerance Indices of Seeds Exposed to C, TW, and Bt-W

Germination Terms	C	TW	Bt-W	Interpretation
Germination Percentage (GP)	94%	81%	90%	Control showed the highest germination in comparison with TW and Bt-W. The lower GP in TW indicates possible toxicity or stress, while Bt-W improved germination compared with TW. (Cokkizgin 2014)
Mean Germination Time (MGT)	3.62	3.70	3.63	TW showed a slightly higher MGT as compared to control, indicating delayed germination. In contrast Bt-W showed an MGT almost identical to the control, suggesting recovery of germination speed after biotreatment. (Rehman <i>et al.</i> 2008; Orchard 1977)
Coefficient of Velocity of Germination (CVG)	27.65	27.0	28.57	Bt-W showed the highest CVG, indicating the fastest and most efficient germination. TW showed the lowest CVG, suggesting slower germination under stress. (Kotowski 1926).
Germination Rate Index (GRI)	29.71	25.32	28.60	Control showed the highest GRI, indicating the fastest germination rate. TW showed a reduced GRI, while Bt-W recovered close to the control level. (Maguire 1962).
Germination Index (GI)	100	71.80	102.49	TW showed reduced GI, indicating moderate inhibition or phytotoxicity. Bt-W had a GI above 100%, indicating improved germination and radicle growth compared with the control (Zucconi <i>et al.</i> 1985).
Radicle length stress tolerance index (RLSTI)	100	126.3	109.0	RLSTI values above 100% in TW and Bt-W indicate enhanced radicle elongation compared with the control. TW showed the highest radicle elongation response, although other indices still indicate stress effects (Rashid <i>et al.</i> 2021).

Shoot length stress tolerance index (SLSTI)	100	83.3	107.1	TW reduced shoot length compared with the control, while Bt-W improved shoot length above the control level, indicating better shoot development after biotreatment (Amin <i>et al.</i> 2013).
Seedling vigor index (SVI)	655.18	567.0	676.80	Bt-W showed the highest seedling vigor, followed by the control, while TW had the lowest SVI. This indicates that biotreated wastewater improved seedling vigor compared with untreated wastewater (Islam and Kato-Noguchi 2014).
Phytotoxicity index (PI)	0	-0.263	-0.090	Negative PI values in TW and Bt-W indicate radicle growth stimulation compared with the control. TW showed stronger radicle elongation stimulation than Bt-W, but this does not mean TW was better overall because other indices showed stress effects (Rashid <i>et al.</i> 2021).

The table summarizes key germination metrics (GP, MGT, CVG, GRI, GI) and derived tolerance indicators (RLSTI, SLSTI, SVI, and PI).

Untreated TW produced relatively high radicle values at some observations, but these did not correspond to similarly improved germination or plumule development. Thus, the percentage of germination, radicle elongation, plumule development, and seedling-vigour indices should be taken together to assess the phytotoxicity of wastewater, rather than on any single growth parameter (Rashid *et al.* 2021). In seed-germination and early growth bioassays, similar decreases in wastewater associated phytotoxicity after biological treatment have been reported (Dhaouefi *et al.* 2019; Selim *et al.* 2021). Overall, the seedling response of Bt-W was closer to the control than untreated TW, which led to the conclusion that biotreatment decreased the phytotoxicity of wastewater.

Germination Behavior of Tomato Seeds

Clear differences in germination parameters were observed among seedlings grown under the control, TW, and Bt-W conditions (Tables 4 and 5). The GP was highest in control with 94%, followed by 90% for Bt-W and 81% for TW, it represented mild inhibitory or phytotoxic effects of untreated textile effluents on seed viability (Zuconi *et al.* 1985; Khan *et al.* 2008). The mean MGT confirmed minimal variation (3.62 to 3.70 days), indicating that all treatments retained similar germination timing; yet the slightly higher MGT in TW indicated a mild delay due to chemical or osmotic stress (Bewley and Black 2013). The CVG and GRI offered an effective measure of vigor with Bt-W showing the highest CVG of 28.6, presenting faster and more effective germination than both the control and TW, which had values of 27.6 and 27.0 respectively (Maguire 1962; Ranal and Santana 2006). This means that Bt-W not only decreased inhibitory compounds but also improved the ionic balance that ultimately resulted in enhanced germination kinetics (Tiquia *et al.* 1996). The GRI result further maintained this trend, with the value of 29.7 for control and 28.6 for Bt-W displaying nearly equivalent rates, while TW (25.3) showed slower rate (Maguire 1962). The germination index (GI) additionally reinforced this observation with Bt-W having 102.5%, slightly exceeding the control baseline of 100%, confirming an overall stimulatory or tolerance-inducing effect, whereas TW with 71.8% presented moderate inhibition, which is typical for industrial wastewater (Qadir *et al.* 2010).

Radicle and Shoot Growth with Stress Tolerance Indices

The radicle length- and plumule length-based stress tolerance indices reflected the capabilities of seedling to tolerate elongation under stressed conditions (Tables 4 and 5). The textile wastewater showed RLSTI of 126.3% with an increase in radicle elongation compared with control and by relating with past studies it is deduced that radicles elongated under stressful conditions to improve water and nutrient requirements (Sharp *et al.* 1988; Comas *et al.* 2013). The Bt-W with RLSTI of 109.0% also enhanced moderate radicle length, confirming positive tolerance, whereas shoot length was reduced in TW with SLSTI of 83.3% and Bt-W of 107.1% which further exceeded the control, signifying that bioremediation improved embryonic shoot growth inhibition and recovered normal growth, which is related with the concept that environmental stress suppressed shoot growth while favourable conditions promoted recovery (Anjum *et al.* 2011; Taiz and Zeiger 2015). The stressed conditions such as salinity influenced radicle elongation by biotreatments of stress-mitigating microbes enhancing tolerance and growth responses (Gupta *et al.* 2017). The overall results suggest that TW led to an irregular growth response, with the radicles growing but the shoot (embryonic) development was reduced. This indicates that the increase in RLSTI in TW may be an adaptive response as a reaction to

stress (Bauer *et al.* 2024). Biotreatment, on the other hand, resulted in greater RLSTI, and a higher SLSTI, suggesting reduced toxic effect of the wastewater, thus favouring the development of plumule and radicle (Miljaković *et al.* 2022). Conclusively, the better seedling growth performance was observed in Bt-W in comparison with TW (Song *et al.* 2023).

Seedling Vigor and Phytotoxicity

The seedling vigor index integrates germination percentage and total seedling length. The SVI was 677 for Bt-W, indicating the most vigorous seedlings, in comparison to 655 for control and 567 for TW. These results indicated that Bt-W not only diminished toxicity but also promoted balanced development of radicle and shoot, reduced toxicity, and improved nutrient accessibility during the process of bioremediation, whereas TW employed an inhibitory effect on early growth (Ranal and Santana 2006). The phytotoxicity index further confirmed these findings, as control exhibited the baseline PI of 0 while both wastewater-treatment groups showed negative PI values of -0.263 and -0.090 for TW and Bt-W respectively, representing a stimulatory effect on radicle elongation compared to the control (Tables 4 and 5). The negative phytotoxicity-index values indicated reduced toxicity or stimulated radicle elongation or that the growth is encouraged, possibly because of available nutrients or adaptive responses activated by mild stress (Khandare and Govindwar 2015; Rizwan *et al.* 2017). Although PI was more negative in TW, its lower SVI indicated that this apparent stimulation of radicle elongation might have been a stress-induced compensatory response instead of an overall enhancement of seedling growth. On the other hand, Bt-W was found to have a high SVI and negative PI, indicating a more balanced and positive impact on seedling metabolism and development. Plant physiological can be destroyed by textile dyes and other related pollutants, which interfere with hormonal balance, specifically auxin, resulting in reduced cell elongation and growth of radicles and shoots (Iqbal *et al.* 2017; Katheresan *et al.* 2018; Ahmad *et al.* 2019).

The radar chart (Fig. 10) is an integrated graphical presentation including comparison between effects of different treatments on the performance of tomato seedlings regarding germination and early growth. Bt-W was found to have a broader and more balanced polygon compared with TW and therefore exhibited better germination behavior, shoot development, and seedling vigor following biotreatment. This implies that bioremediation minimized the toxicity of TW and may have enhanced nutrient availability, facilitating the better physiological growth of seedlings (Khandare and Govindwar 2015; Rizwan *et al.* 2017; Ahmad *et al.* 2019). The control presented a moderate and balanced growth profile which represents normal physiological growth under tap water. The partial overlap of control and Bt-W suggests that the biotreated sample produced growth responses comparable to, and for certain parameters greater than, the control condition. Conversely, TW showed comparatively poorer performance for germination percentage, germination index, shoot length, and seedling vigor, which may reflect the inhibitory effects of salts, dyes, or heavy metals on enzyme activity, water uptake, and physiological growth processes (Munns and Tester 2008; Qadir *et al.* 2010). Although TW showed comparatively greater radicle elongation for some measurements, this may represent a stress-associated root response rather than an overall improvement in seedling performance. The radar profile integrates the different germination and stress-response indices and illustrates a more balanced overall response under Bt-W than TW (Fig. 10). Rather than demonstrating a specific pollutant removal mechanism, this biological response provides complementary evidence that the treated wastewater exerted lower

phytotoxic effects than the untreated wastewater. The result is consistent with previous research showing that seed germination and root elongation assays provide sensitive biological measures of wastewater phytotoxicity, complementing physicochemical measurements that may not fully capture potential effects on plants (Priac *et al.* 2017).

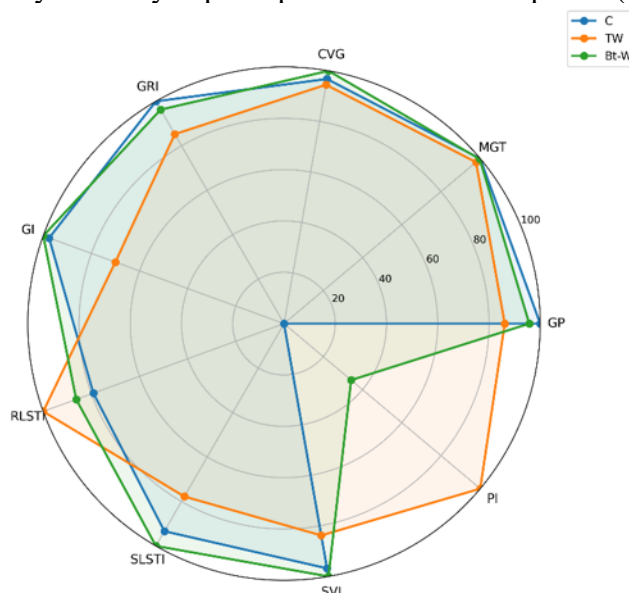


Fig. 10. Radar chart showing the normalized germination and stress-tolerance profile of seedlings under control (C), TW, and Bt-W treatments. Values were normalized to a 0–100 scale to allow comparison among germination, growth, vigor, and phytotoxicity-related parameters. Higher scores indicate better performance, except that MGT and PI were interpreted as a phytotoxicity-related response rather than a direct indicator of better performance.

It should be noted that the 8th day laboratory scale batch treatment period does not represent the extended microbial acclimation and community stabilization periods typically observed in full scale wastewater treatment systems. Industrial activated sludge and anaerobic reactors benefit from long term biomass retention, recycling, and continuous selection pressures that allow highly adapted microbial communities to develop (Chen *et al.* 2017; Dottorini *et al.* 2023). Therefore, the present study evaluates the short term treatment potential of a selected indigenous bacterial consortium under controlled laboratory conditions rather than replicating the mature microbial ecology of industrial wastewater treatment facilities. Future studies should investigate longer operational periods, continuous flow reactor configurations, repeated treatment cycles, and microbial community dynamics to assess long-term stability and adaptation of the consortium.

CONCLUSIONS

This study was conducted under controlled laboratory conditions to evaluate the treatment potential of a selected indigenous bacterial consortium for textile wastewater remediation. The findings should be interpreted within the scope of the applied experimental conditions and represent the observed treatment responses under the investigated system rather than direct validation of full-scale wastewater treatment performance. Further studies under larger-scale and continuous treatment conditions are required to assess long-term performance and practical applicability.

1. Under the laboratory-scale batch conditions investigated, the selected indigenous bacterial consortium improved several measured characteristics of textile wastewater evaluated on the 8th day treatment period. These findings demonstrate the treatment potential of the consortium under the applied experimental conditions.
2. Biological treatment reduced the measured aqueous concentrations of several heavy metals and improved the overall physicochemical characteristics of the wastewater. However, these reductions represent laboratory scale treatment responses and do not by themselves establish complete contaminant removal or suitability for unrestricted reuse.
3. The tomato seed bioassay showed an improved overall response following biotreatment. Biotreated wastewater (Bt-W) had the highest seedling vigor index (677), followed by the control (655) and untreated textile wastewater (TW) (567), while its germination and plumule-development responses were also closer to the control. These findings support a reduction in wastewater-associated phytotoxicity following biological treatment.
4. Untreated TW produced relatively high radicle elongation at some observation points, but this was not accompanied by similarly improved germination or plumule development. Radicle elongation alone should therefore not be interpreted as evidence of improved overall seedling performance, and the greater radicle growth observed under TW may reflect a stress associated response.
5. Overall, the findings indicate that bacterial treatment can improve measured wastewater-quality characteristics and reduce phytotoxic effects under controlled laboratory conditions. However, the present results do not establish that the treated wastewater is safe or suitable for routine agricultural irrigation under field conditions. Rather, they indicate its potential for further evaluation for irrigation reuse.
6. Further studies should include multiple independent bioreactor runs, pilot-scale or continuous treatment systems, and field-based irrigation experiments. Long-term effects on soil properties, plant growth, crop productivity, residual contaminant accumulation, and other relevant irrigation water quality parameters should also be assessed before practical agricultural reuse can be recommended.

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Competing Interests

The authors declare that there are no conflicts of interest.

Availability of Data and Material

All the data generated in this research work have been included in this manuscript.

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