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Bacterial bioremediation: A sustainable approach to wastewater treatment and promoting the growth of *Vicia faba* (L.)

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This study explores the use of bacterial isolates from wastewater treatment plants in El-Gharbia Governorate, Egypt, for improving wastewater quality and enhancing the growth of *Vicia faba* (L.) seedlings. Twenty-five bacterial isolates were collected from various wastewater treatment stages, including influent, sedimentation tanks, aeration tanks, and effluents. Five isolates (1, 3, 7, 15, and 21) were identified as highly effective in treating wastewater whereas, isolate no.1 achieved the most significant reductions in total suspended solids (TSS), biological oxygen demand (BOD), and chemical oxygen demand (COD). Further testing on Kafr El-Zayat wastewater showed that isolate No. 1 had ability to reduce TSS, BOD, and COD by 60%, 61%, and 68%, respectively, after two days. A mixture of the top five isolates (Tmix) improved these reductions to 65%, 68%, and 70%. Furthermore, the bacterial consortium effectively removed heavy metals such as Cd, Cr, Cu, Fe, Mn, and Pb, with removal efficiencies up to 97% for Mn and 96% for Fe. Molecular identification using 16S rRNA sequencing identified the isolates as *Klebsiella sp., Proteus sp., Trabulsiella sp., Escherichia sp.,* and *Kosakonia sp.* Irrigation with treated wastewater, especially using Tmix, significantly improved the growth of *Vicia faba* (L.) seedlings, enhancing shoot height, fresh weight, photosynthetic efficiency, antioxidant enzyme activities, and key biochemical compounds like ascorbic acid, phenolics, and flavonoids. The treatments also increased nutrient uptake, particularly potassium and nitrogen. This study highlights bacterial bioremediation as a sustainable approach to wastewater treatment and agricultural application.

Keywords: Wastewater treatment, Heavy metal removal, Removal efficiency, *Vicia faba* (L.) Sustainable agriculture

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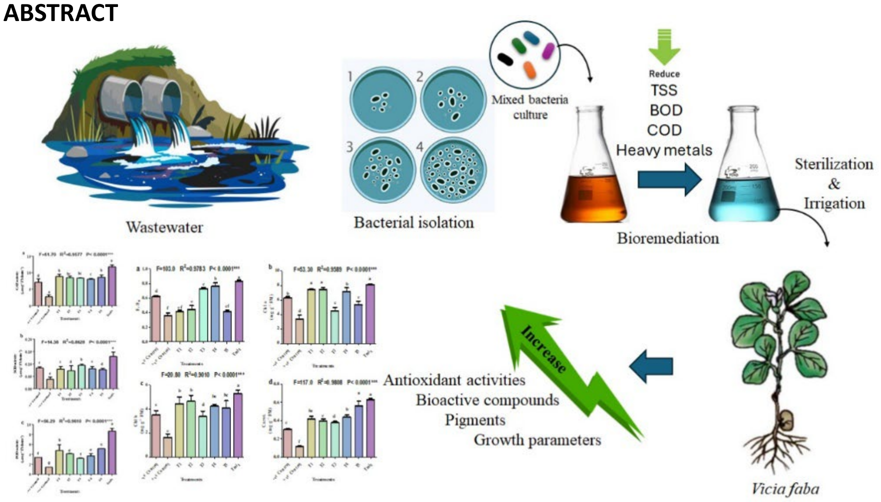
GRAPHICAL ABSTRACT



INTRODUCTION

Water is a vital natural resource, indispensable for sustaining life, supporting industrial development, and ensuring agricultural productivity. Access to clean and sufficient freshwater is particularly critical for developing nations, as it underpins stable communities and economic growth (Tella *et al*., 2025). However, the escalating global population, rapid industrialization, and the impacts of climate change have exacerbated water scarcity and pollution, posing significant challenges to sustainable development. In this context, wastewater treatment has emerged as a crucial strategy to mitigate these issues by reducing water waste, alleviating pressure on freshwater resources, and promoting environmental sustainability (Silva, 2023).

Wastewater treatment not only removes pollutants, coarse particles, and toxic substances but also generates valuable byproducts such as bio-methane and nutrient-rich manure, which can be utilized in agriculture (Thakur *et al.,* 2025). Furthermore, treated wastewater increases water availability for reuse, offering a practical solution to the growing global water crisis (Kahn *et al*., 2025).Human activities, including agriculture, industrial discharges, and domestic effluents, introduce harmful pollutants such as organic matter, heavy metals, and pathogens into aquatic ecosystems. These contaminants disrupt ecological balance, threaten biodiversity, and pose severe risks to human health (Iyiola *et al*., 2024). Wastewater treatment typically involves a multi-stage process: preliminary treatment removes large solids and debris to prevent equipment damage primary treatment separates suspended solids and floating substances; secondary treatment reduces biochemical oxygen demand (BOD) and chemical oxygen demand (COD) by decomposing organic matter using microorganisms and tertiary or advanced treatment removes nutrients, heavy metals, and microbiological contaminants through methods such as chlorination, ozonation, or ultraviolet treatment (Wu *et al*., 2025). Among these, biological treatment stands out as an effective and economical approach, relying on microorganisms like bacteria, fungi, and algae to degrade organic pollutants and remove heavy metals (Wang *et al*., 2024a).Biological treatment can be categorized into aerobic, anaerobic, and facultative processes.

Aerobic treatment utilizes oxygen-dependent microorganisms to break down organic matter and is commonly applied in activated sludge systems and trickling filters (Liang *et al*., 2024). Anaerobic treatment, on the other hand, occurs in oxygen-free environments where microorganisms degrade organic matter, producing methane as a byproduct, and is typically used in anaerobic digesters. Facultative treatment combines aerobic and anaerobic processes, offering flexibility in treating wastewater with varying characteristics (Wang *et al*., 2024b). Bacteria play a pivotal role in biological wastewater treatment due to their remarkable metabolic versatility and adaptability. These microorganisms can degrade complex organic compounds into simpler, less harmful substances, transforming pollutants into water, carbon dioxide, and biomass (Aragaw, 2021). This process is facilitated by enzymes produced by bacteria, which target specific contaminants, enabling efficient breakdown and detoxification. Additionally, bacteria can adsorb heavy metals onto their cell walls or metabolize them through various biochemical pathways, reducing their toxicity and environmental impact (Sarkar and Bhattacharjee, 2025). The adaptability of bacteria to diverse environmental conditions further enhances their utility in wastewater treatment. Aerobic bacteria require oxygen to metabolize organic pollutants, while anaerobic bacteria operate in oxygen-deprived environments, breaking down organic matter and producing methane. Facultative bacteria can switch between aerobic and anaerobic modes, making them highly adaptable to fluctuating wastewater conditions (Yu *et al*., 2023).

The use of bacterial consortia, which combines multiple species of bacteria, significantly enhances treatment efficiency. This synergistic approach allows for a division of labor, where each species targets specific pollutants (Chen *et al*., 2020). For instance, one bacterium might specialize in degrading hydrocarbons, while another excels at nitrification or denitrification. Moreover, the metabolic byproducts of one species can serve as substrates for another, creating a mutually beneficial network that optimizes biodegradation and pollutant removal (Singh *et al*., 2022a). Bacterial consortia are particularly effective in addressing complex contaminants, including recalcitrant compounds and heavy metals. Through bio-adsorption, bacteria can bind heavy metals such as cadmium (Cd), lead (Pb), and chromium (Cr) onto their cell walls, aided by surface molecules like proteins, lipopolysaccharides, and extracellular polymeric substances (EPS). These EPS not only enhance adsorption capacity but also protect bacterial cells from the toxic effects of heavy metals. Furthermore, bacterial consortia demonstrate resilience under varying environmental conditions, such as changes in pH, temperature, and pollutant load (Sarkar and Bhattacharjee, 2025). Research has shown that bacterial consortia outperform single-species treatments in terms of pollutant removal efficiency and process stability (Mahapatra *et al*., 2020).These advancements highlight the potential of bacteria as a sustainable and cost-effective solution for improving wastewater quality and safeguarding aquatic ecosystems. Future research should focus on scaling up these technologies, exploring novel bacterial strains, and integrating them with emerging treatment methods to address the growing challenges of water pollution and resource recovery. In the context of sustainable agriculture, the reuse of treated wastewater offers dual benefits: providing a nutrient-rich irrigation resource and reducing environmental pollution. However, stringent guidelines from international organizations like the World Health Organization are essential to ensure safe agricultural use and minimize health risks associated with pathogens (Rafi *et al*., 2025).The faba bean (*Vicia faba* L.) is a prominent legume crop, particularly in Egypt, valued for its high protein content (30%), dietary fibers, essential amino acids, carbohydrates, and minerals. It serves as a significant dietary staple and plays a vital role in sustainable agriculture (Dhull *et al*., 2022). As a nitrogen-fixing legume, *Vicia faba* (L.) enhances soil fertility, improving the productivity of subsequent crops in rotational farming systems. Its adaptability to wastewater irrigation makes it a promising candidate for evaluating the agricultural use of treated wastewater. Studies have shown that nutrient-rich treated wastewater can promote *Vicia faba* (L.) growth, enhance photosynthetic efficiency, and increase the biochemical composition of the plant, including phenolics, flavonoids, and antioxidants (Mohamed *et al*., 2019).

This study adopts a biotechnological approach to treat wastewater in the El-Gharbia Governorate using bacterial isolates capable of reducing COD, BOD, and TSS. Additionally, it investigates the effects of biologically treated wastewater on the growth and biochemical characteristics of *Vicia faba* (L.) seedlings, aiming to contribute to the sustainable reuse of treated wastewater in irrigation.

MATERIALS AND METHODS

Bacterial isolation

This study was conducted from June to August 2022, during which water samples were collected from three different stages of wastewater treatment plants in El-Gharbia Governorate, Egypt, specifically in Delbshan, Kafr El-Zayat, and Tanta. The Universal Transverse Mercator (UTM) coordinates are: 288818.6623°N, 3408065.483°E; 293007.155°N, 3411933.321°E; and 310529.0055°N, 3409546.417°E. Sampling points included influent, primary and secondary sedimentation tanks, and effluent stages. One-liter water samples were collected in sterile polypropylene bottles with 2.5 cm of airspace, transported on ice to the laboratory, and processed within 24 hours. Serial dilutions were made using sterile normal buffer solutions, and 1 mL aliquots were plated on nutrient agar, a general-purpose medium for bacterial growth (Zimbro *et al*., 2009).

Plating method

Sterilized nutrient agar was poured into Petri dishes (4 mm thickness) under aseptic conditions in a laminar flow hood. Once solidified and dried (15–30 minutes), samples were streaked in a zigzag pattern to ensure uniform distribution on the agar surface. Plates were incubated at 37°C for 24 hours for bacterial growth (Zimbro *et al*., 2009).

Bacterial purification and morphological identification

Individual bacterial colonies were selected based on cultural characteristics and colony morphology. These colonies were sub-cultured on fresh nutrient agar plates and incubated overnight at 37°C for pure cultures. Morphological features, including colony color, shape, Gram reaction, and margins, were documented for further identification (Dimri *et al*., 2020).

Molecular identification of bacterial isolates using 16S rRNA

Pure isolates were cultured in nutrient broth at 37°C for 48 hours (Zimbro *et al*., 2009). DNA extraction and 16S rRNA sequencing were performed in MacroGen Co., South Korea using universal primers 27F and 1492R (Elshobary *et al*., 2015). Sequencing results were verified by electrophoresis on 1% agarose gel and phylogenetic tree was analyzed with Neighbor joining method, BLAST Tree View software.

Evaluation of bacterial isolates for wastewater treatment

Various purified bacterial isolates were assessed for their potential to treat wastewater by analyzing several parameters in the wastewater after 24 hours of incubation. The most effective isolates, which showed a significant reduction in all measured parameters, were selected for further identification and detailed investigation.

Preparation of bacterial inoculum for wastewater treatment

The top five bacterial isolates were maintained on nutrient agar at 4°C and sub-cultured biweekly. Each isolate was cultured in nutrient broth (50 mL) in 250 mL flasks at 30°C for 24 hours on a shaker (120 rpm). The bacterial growth of five selected bacteria isolates was assessed by colony count to adjust the number of cells to 1×10^8 CFU. A specific volume of cultures or inoculum was poured into a centrifuge tube under aseptic conditions, centrifuged at 3000 rpm for 10 minutes, and pellets were suspended in 400 mL sterile wastewater. Treated samples were incubated at 30°C for 2 and 4 days for analysis (El-Gammal, 2003).

Determination of total dissolved solids (TDS)

The total dissolved solids (TDS) of the water samples were measured using a conductivity meter to evaluate the removal efficiency of TDS in wastewater (Igwegbe and Onukwuli, 2019). The TDS concentration was recorded before treatment (TDS_i) and after treatment (TDS_f). The removal efficiency was calculated using the formula: $TDS (\%) = [(TDS_i - TDS_f) / TDS_i] \times 100$.

Determination of total suspended solids (TSS)

The concentration of total suspended solids (TSS) was measured before treatment (TSS_i) and after treatment (TSS_f) to determine removal efficiency (Verma *et al*., 2013). The removal efficiency was calculated as follows: TSS (%) = $[TSS_i - TSS_f] / TSS_i] \times 100$

Measurement of pH

The pH of water samples was measured using a pH meter. The pH meter was calibrated, and its electrode was rinsed with distilled water before being dipped 2 to 3 cm into the sample. The sample was stirred once, and the reading was allowed to stabilize before recording.

Determination of biological oxygen demand (BOD)

BOD removal efficiency in wastewater was assessed by measuring its concentration before treatment (BOD_i) and after treatment (BOD_f) (Abdalla and Hammam, 2014). The removal efficiency was calculated as: BOD (%) = $[(BOD_i - BOD_f) / BOD_i] \times 100$

Determination of chemical oxygen demand (COD)

COD removal efficiency was determined by measuring its concentration before treatment (CODi) and after treatment (CODf) (Kadhum *et al*., 2021). The removal efficiency was calculated using the formula:

COD (%) = $[(COD_i - COD_f) / COD_i] \times 100$.

Determination of heavy metals in wastewater

Heavy metal concentrations (Cd, Cu, Cr, Fe, Mn, Pb) were measured using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Vista Pro). Samples (100 mL) were acidified with 10 mL of concentrated HNO3, boiled to reduce volume, and digested as described by Srikanth *et al*. (2014). The removal efficiency was calculated as:

Removal Efficiency (%) = $[(C_i - C_e) / C_i] \times 100$

Where C_i is the concentration of heavy metal element in wastewater and C_e is the concentration of heavy metal element after biological treatment (Kumar and Chopra, 2012).

The growth conditions and biochemical analysis of *Vicia faba* (L.)

Plant material

Faba bean seeds (*Vicia faba* L., cv. Giza 3) were obtained from the Sakha Research Station, Kafr El-Sheikh, Agriculture Research Center, Department of Agronomy, Egypt. Seeds were disinfected with 1% NaClO for two minutes and rinsed three times with sterile distilled water.

The growth conditions of *Vicia faba* (L.)

Seeds of *Vicia faba* were divided into three groups: Negative control: Irrigated with tap water. Positive control: Irrigated with untreated sterilized wastewater. Experimental groups: Subdivided into six groups irrigated with treated sterilized wastewater using: T1: *Proteus* sp. Isolate, T2: *Klebsiella* sp. Isolate, T3: *Trabulsiella* sp. Isolate, T4: *Escherichia* sp. Isolate, T5: *Kosakonia* sp. Isolate, Tmix: Mixture of T1, T2, T3, T4, and T5. Five seeds per group were sown in 15 cm diameter and 20 cm depth plastic pots filled with 5 kg clay-sandy soil (2:1 w/w). Pots were arranged in a randomized complete block design with three replicates (24 pots total). During the first week, plants were irrigated with tap water until emergence. Thereafter, plants were irrigated twice weekly with the designated treatments. Negative controls received tap water, and positive controls received wastewater.

Growth parameters

On 21 days, seedlings were harvested and separated into roots, shoots, and leaves. Fresh weight (FW) and dry weight (DW) were measured. Growth parameters included shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), plant height, and leaf area.

Photosynthetic efficiency and pigments

Photosynthetic activity was assessed using the OS-30p chlorophyll fluorometer. Chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids were extracted and quantified as mg g^{-1} FW according to Arnon (1949) and Horvath *et al*. (1972).

Antioxidant enzyme activity

According to Beauchamp and Fridovich (1971), 0.5 g of fresh plant material was frozen and then homogenized in 8 ml of 50 mM cold phosphate buffer with a pH of 7.0. For 20 minutes, the homogenates were centrifuged at 4000 rpm. For the enzymatic test, the supernatant was utilized as a raw extract. The decrease in absorbance at 240 nm caused by H_2O_2 degradation (extinction coefficient 36×10^3 mM^{-1} cm^{-1}) in 20 mM H_2O_2 and 50 mM potassium phosphate buffer (pH 7) was used to measure the activity of catalase (CAT, EC 1.11.1.6) (Kato and Shimizu, 1987). Superoxide dismutase (SOD, EC 1.15.1.1) activity was assessed by preventing nitro blue tetrazolium (NBT) from being photochemically reduced at 560 nm.75 µM NBT, 100 µM EDTA, 2 µM riboflavin, 13 mM L-methionine, and 50 mM potassium phosphate buffer (pH 7.8) were all present in the reaction mixture. The molar extinction coefficient of 21.1 mM^{-1} cm^{-1} to produce blue formazan was used to express the activity of SOD (van Rossum *et al*., 1997). Guaiacol peroxidase (POD, EC 1.11.1.7) activity was measured in accordance with Kato and Shimizu (1987). 50 mM potassium phosphate buffer (pH 5.8), 7.2 mM guaiacol, 11.8 mM H_2O_2, and 0.1 mL enzyme extract were all present in the 1 mL combination. Guaiacol oxidation caused an increase in absorbance at 470 nm (extinction coefficient 26.6 mM^{-1} cm^{-1}).

Total soluble carbohydrates and proteins

The phenol-sulfuric acid method was used to measure the total amount of soluble sugars (Dubois *et al*., 1956). 5 mL of borate buffer was mixed with 0.1 g of fine plant powder. After a 24-hour rest period, the mixture was centrifuged for 15 minutes at 3000 rpm. The mixture is then quickly mixed with a solution that contains 0.1 mL of borate buffer, 1 mL of phenol (5%), and 5 mL of strong sulfuric acid (95%). The test tubes are vortexed for 30 seconds after standing for 10 minutes, and then they are left in a water bath at room temperature for 20 minutes to develop their color. A spectrophotometer is then used to record light absorption at 490 nm. The amount of total soluble protein was calculated using Bradford's methodology. 3 ml of Coomassie Brilliant Blue (CBB) reagent (100 mg of CBB was dissolved in 50 ml of 95% ethanol and 100 ml of 85% phosphoric acid) was well mixed with precisely 0.1 ml of borate buffer extract before being added to one liter of distilled water. The absorbance at 595 nm was measured after two minutes. Using a calibration curve of bovine serum albumin as a standard protein, the concentration of total soluble proteins was calculated as mg/g DW.

Phenolics, flavonoids, and ascorbic Acid

The Folin-Ciocalteu colorimetric method, as outlined by Mongkolsilp *et al*. (2004), was used to determine the total phenolic content. The reaction mixture contained 2.5 mL of Folin-Ciocalteu reagent, 2 mL of 7.5% sodium carbonate solution, 0.5 mL of sample extract (0.1 g of the dried leaf tissues were extracted by 95% ethanol for 24 hours, and then filtered while the clear supernatants were obtained and completed to a known volume of 10 ml), and it was incubated for 30 minutes at room temperature before the absorbance at 750 nm was measured. Zhishen *et al*. (1999) technique was used to assess the total flavonoid content. In short, 0.4 mL of distilled water, 0.3 mL of a 10% sodium nitrite solution, and 1 mL of the plant extract were combined, and the combination was allowed to stand for five minutes. Next, two milliliters of 1% sodium hydroxide and 0.3 milliliters of a 10% aluminum chloride solution were added. The absorbance was measured at 510 nm. The ascorbic acid content was assessed according to Oser (1979). After homogenizing 0.1 g of fresh leaves in 5 ml of 5% w/v sulfosalicylic acid, the sample was centrifuged for 10 minutes at 10,000 rpm. 2 ml of 2% Na-molybdate, 2 ml of H2SO4 (0.15 N), 1 ml of 1.5 mM Na2HPO4, and 1 ml of leaf extract were all present in the homogenates. After 40 minutes of incubation at 60°C in a water bath, the mixture was cooled and centrifuged for 10 minutes at 3000 rpm. The absorbance was measured at 660 nm.

Minerals content of *Vicia faba* (L.)

Dried plant samples were digested with a mixture of nitric and perchloric acids. Elemental analysis was performed using Atomic Absorption Spectrophotometer (Allen et al., 1974). Phosphorus was determined using the molybdenum blue method, and nitrogen was estimated via phenol-sodium nitroprusside reaction with spectrophotometric detection.

Statistical analysis

Data were analyzed as means ± standard deviation (SD) for replicates. Duncan's test was used for comparing means at a 5% significance level using SPSS 15 software.

RESULTS

Isolation of bacteria from wastewater

Twenty-five bacteria isolates were isolated from three wastewater plants (Delbshan, Kafr El-Zayat and Tanta) in El-Gharbia Governorate, Egypt. Samples were collected from different sites in plants representing the influent, primary and secondary sedimentation tanks, aeration tanks and the effluent (Figure S1). Bacteria samples petrified on nutrient agar media after serial dilution of water sample (Figure S2). The obtained data in Figure S3 show variable morphological characteristics of pure bacterial isolate on a nutrient agar medium. The isolated bacteria were selected and purified according to different morphological characteristics such as colour, opacity, shape (form), margin and gram stain then the isolated bacteria were tested to investigate their ability to treat wastewater (Table 1).

Identification of selected bacterial isolates 16S rRNA gene

The results of identification indicated in Figure 1 and Table S1 revealed that applying the biosystem 16S ribosomal RNA sequence showed that the tested bacterial isolates exhibited a similarity of 98% to the 16S ribosomal RNA sequence of *Klebsiella sp* and showed a similarity of 99% for both *Proteus sp.* with accession NO (OR976477), *Trabulsiella sp.* with accession NO (OR984189), *Escherichia sp.* with accession NO (OR984151), and *Kosakonia sp.* with accession NO (PP029192) respectively. The partial nucleotide sequence of the 16s rDNA gene for *Klebsiella sp, Proteus sp., Trabulsiella sp., Escherichia sp.,* and *Kosakonia sp.* isolates was done to determine the relationship with other recommended 16s DNA gene of previous strains registered in GenBank (Figure 1 & Table S1).

Investigation of the ability of bacterial isolate to treat wastewater

Different purified bacterial isolates were evaluated for their ability to treat wastewater by measuring several parameters, including total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), Total Dissolved Solids (TDS), and pH, after 24 hours of incubation. The results, as shown in Figure 2, indicates that isolates no. 1, 3, 7, 15, and 21 had a significant efficiency in treating wastewater. All measured parameters: total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), and pH showed a notable decline compared to untreated wastewater and other bacterial isolates. The measured values for the top-performing isolates were as follows: TSS levels ranged between 52±0.16 and 99±0.19 mg/L, BOD between 103±0.19 and 126±0.38 mg/L, COD between 85±0.26 and 117±0.47 mg/L, TDS between 1466±7.33 and 1539±10.77 mg/L, and pH between 8.29±0.02 and 8.51±0.02. These findings highlight the most substantial and effective improvements in wastewater quality achieved by these isolates, outperforming the remaining 25 purified bacterial strains (Figure 2).

Removal efficiency of TSS, BOD, COD and TDS from treated wastewater

The data presented in Table 2 reveals clear differences in the removal efficiency of TSS, BOD, COD, and TDS across all tested bacterial treatments (T1–T5) and their mixture (Tmix), evaluated after 2 and 4 days of incubation. Upon comparison, results revealed that after 2 days the Tmix shows the highest removal efficiency across all parameters whereas, the percentages of removal efficiency of TSS, BOD, COD, and TDS were 65%, 68%,70%, and 38%, respectively. These values are significantly higher than those achieved by individual treatments (T1–T5). Among the individual treatments, T1 consistently performs better than the others, with values of 60%, 61%, 68%, and 34% for TSS, BOD, COD, and TDS, respectively. T5 showed the lowest removal efficiency for all parameters, indicating limited effectiveness as a standalone treatment. Moreover, after 4 Days, Tmix maintains its superior performance, with the highest removal efficiencies: TSS (97%), BOD (95%), COD (98%), and TDS (50%). Results indicated that the synergistic effect of combining bacterial strains is sustained and becomes more pronounced over time.

Individual treatments (T1–T5) shows improvements after 4 days, but their efficiencies remain lower than Tmix. Also, T1 achieves 95% TSS, 92% BOD, 94% COD, and 45% TDS, which is the highest among individual strains but still below Tmix. T5, on the other hand, showed the lowest efficiency after 4 days, with values of 90%, 91%, 92%, and 43%, respectively. The comparison of removal efficiencies clearly establishes Tmix as the most effective treatment for sterilized wastewater, with significant advantages over individual treatments.

The effect of treated wastewater on the content of heavy metals

Table 3 demonstrated the effectiveness of bacterial isolates (T1–T5) and their mixture (Tmix) in reducing heavy metal concentrations (Cd, Cr, Cu, Fe, Mn, Pb) in wastewater over 2 and 4 days. The mixture treatment (Tmix) consistently achieving the lowest levels for all tested metals (Cd, Cr, Cu, Fe, Mn, Pb) after both 2 and 4 days of incubation. After 2 days, Tmix drastically reduced Fe (0.839 mg/L), Mn (0.084 mg/L), and Pb (0.030 mg/L) compared to untreated wastewater and individual treatments. After 4 days, Tmix continued to outperform all other treatments, with the lowest Fe (0.128 mg/L), Mn (0.041 mg/L), and Pb (0.066 mg/L) levels. These results highlight the synergistic effect of combining bacterial isolates, making Tmix a promising candidate for bioremediation of wastewater.

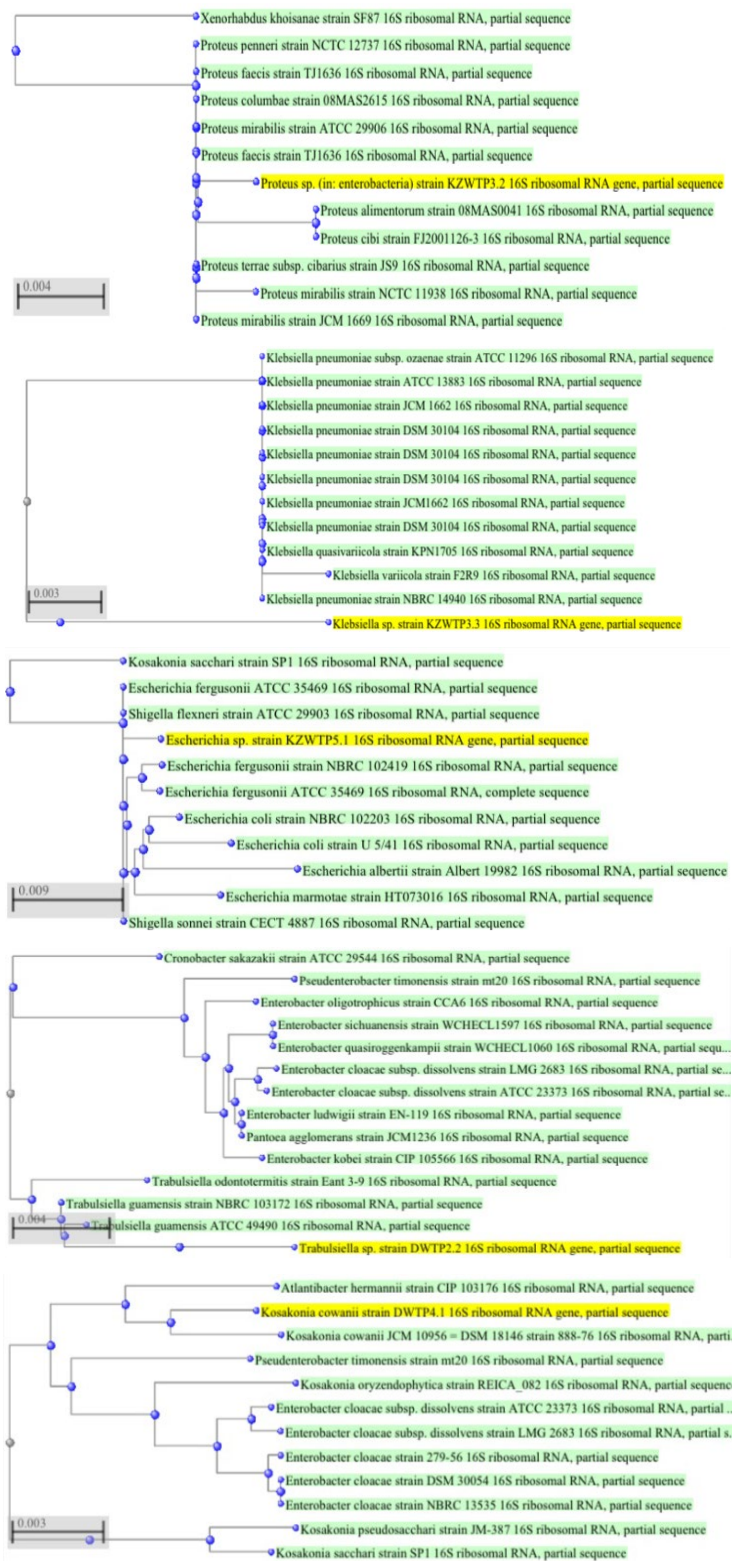


Figure 1. Neighbor-joining trees indicating phylogenetic relationships of the five most potent selected bacterial isolates from wastewater and their nearest relatives based on GenBank 16S rRNA sequences.

Table 1. The morphological characterization of the bacterial isolated from samples of wastewater

No.	Color	opacity	Shape (form)	margin	Gram reaction	Shape of cells
1	Dark yellow	Opaque	Round	Entire	G-ve	Rod shape
2	White	Translucent	Irregular	Entire	G-ve	Rod shape
3	Pink	Opaque	Round	Entire	G-ve	Rod shape
4	White	Opaque	Round	Lobate	G+ve	Cocci
5	Golden yellow	Opaque	Round	Entire	G-ve	Cocci
6	Pale white	Transparent	Round	Undulate	G+ve	cocci
7	Yellow	Opaque	Round	Entire	G-ve	Rod shape
8	White	Translucent	Round	Entire	G+ve	Bacilli
9	White	Opaque	Round	Undulate	G-ve	Rod shape
10	Orange	Opaque	Round	Entire	G-ve	Rod shape
11	White	Transparent	Round	Entire	G-ve	Rod shape
12	Golden yellow	Translucent	Round	Lobate	G+ve	Cocci
13	Red	Opaque	Round	Entire	G-ve	Rod shape
14	Pale white	Translucent	Round	Entire	G-ve	Rod shape
15	White	Opaque	Round	Entire	G-ve	cocci
16	White	Transparent	Irregular	Curled	G+ve	cocci
17	Grey	Transparent	Round	Entire	G-ve	bacilli
18	Pale yellow	Translucent	Round	Entire	G+ve	Rod shape
19	Orange	Translucent	Round	Entire	G+ve	cocci
20	Off white	Opaque	Round	Curled	G-ve	Rod shape
21	Pale yellow	Opaque	Round	Entire	G-ve	Bacilli
22	Off white	Transparent	Irregular	Entire	G+ve	cocci
23	Yellow	Translucent	Round	Lobate	G-ve	Rod shape
24	White	Transparent	Filamentous	Filiform	G-ve	Rod shape
25	White	Opaque	Irregular	Lobate	G+ve	cocci

Table 2. The percentage removal efficiency of TSS, BOD, COD and TDS from sterilized wastewater treated with *Proteus sp.* (T1), *Klebsiella sp.* (T2), *Trabulsiella sp.* (T3), *Escherichia sp.* (T4), *Kosakonia sp*. (T5), and mixture of T1,T2,T3,T4,T5 (Tmix) after 2 and 4 days of their incubation

Days of Incubation	Water Samples	TSS (%)	BOD (%)	COD (%)	TDS (%)
After 2 days	T1	60 ± 0.42^{b}	61 ± 0.52^{b}	68 ± 0.44^{a}	34 ± 0.36^{b}
	T2	55 ± 0.45^{c}	51 ± 0.50^{c}	60 ± 0.43^{b}	33 ± 0.41^{b}
	T3	57 ± 0.41^{c}	57 ± 0.56^{c}	63 ± 0.51^{b}	30 ± 0.44^{c}
	T4	49 ± 0.39^{d}	49 ± 0.46^{d}	57 ± 0.56^{c}	34 ± 0.55^{b}
	T5	47 ± 0.50^{d}	46 ± 0.43^{d}	53 ± 0.49^{d}	31 ± 0.37^{c}
	Tmix	65 ± 0.44^{a}	68 ± 0.51^{a}	70 ± 0.45^{a}	38 ± 0.39^{a}
	R^2	0.9912	0.9932	0.9832	0.9732
	P	***	***	***	***
After 4 days	T1	95 ± 0.40^{b}	92 ± 0.29^{b}	94 ± 0.50^{b}	45 ± 0.33^{b}
	T2	91 ± 0.37^{d}	93 ± 0.49^{b}	92 ± 0.42^{c}	40 ± 0.45^{d}
	T3	93 ± 0.45^{c}	91 ± 0.37^{c}	95 ± 0.56^{b}	39 ± 0.40^{e}
	T4	92 ± 0.44^{c}	90 ± 0.63^{c}	93 ± 0.38^{c}	44 ± 0.61^{b}
	T5	90 ± 0.29^{d}	91 ± 0.50^{c}	92 ± 0.47^{c}	43 ± 0.56^{c}
	Tmix	97±0.51a	95 ± 0.27^{a}	98 ± 0.33^{a}	50 ± 0.38^{a}
	R^2	0.9285	0.9644	0.9941	0.9935
	P	***	***	***	***

Removal efficiency of heavy metals (Cd, Cr, Cu, Fe, Mn and Pb) in treated wastewater

Table 4 presented the percentage removal efficiency of heavy metals (Cd, Cr, Cu, Fe, Mn, Pb) from sterilized wastewater treated with individual bacterial isolates (T1–T5) and their mixture (Tmix) after 2 and 4 days of incubation. The data revealed that the bacterial mixture (Tmix) consistently showed the highest removal efficiency for most metals after both incubation periods. After 2 days, Tmix achieved the highest removal rates for Cd (50%), Cu (73%), and Pb (70%), and had a relatively high performance for Cr (47%), Fe (87%), and Mn (93%). It outperformed individual isolates such as T2 for Cr and T5 for Mn and Pb, demonstrating a strong combined effect of the bacterial isolates. The individual treatments varied, with T2 showing the best removal efficiency for Cr (67%) and T5 for Mn (86%), while T1 consistently showed the lowest removal efficiency across all metals. After 4 days, the mixture continued to perform well, achieving high removal rates across all metals (Cd: 91%, Cr: 85%, Cu: 87%, Fe: 96%, Mn: 97%, Pb: 73%). T5 showed the best performance for Cd (87%), Cr (92%), Cu (83%),

Table 3. The content of heavy metals (Cd, Cr, Cu, Fe, Mn and Pb) in wastewater and sterilized wastewater treated with *Proteus sp.* (T1), *Klebsiella sp.* (T2), *Trabulsiella sp.* (T3), *Escherichia sp.* (T4), *Kosakonia sp*. (T5), and mixture of T1,T2,T3,T4,T5 (Tmix) after 2 and 4 days of their incubation

Days of Incubation	Water Samples	Cd (mg/L)	Cr (mg/L)	Cu (mg/L)	Fe (mg/L)	Mn (mg/L)	Pb (mg/L)
After 2 days	wastewater	0.006±0.0003^{a}	0.030±0.003^{a}	0.051±0.0046^{a}	6.247±0.375^{a}	1.198±0.084^{a}	0.093±0.007^{a}
	T1	0.005±0.0004^{b}	0.021±0.0021^{b}	0.040±0.0032^{b}	1.499±0.075^{b}	0.506±0.035^{c}	0.064±0.003^{b}
	T2	0.005±0.0005^{b}	0.010±0.0008^{e}	0.019±0.0015^{c}	0.622±0.025^{d}	0.434±0.026^{d}	0.043±0.003^{d}
	T3	0.004±0.0002^{c}	0.019±0.0013^{b}	0.036±0.0036^{b}	1.641±0.082^{b}	0.708±0.035^{b}	0.052±0.001^{c}
	T4	0.004±0.0004^{c}	0.014±0.0008^{d}	0.015±0.0009^{d}	1.035±0.052^{b}	0.158±0.008^{e}	0.059±0.002^{c}
	T5	0.005±0.0001^{b}	0.014±0.0007^{d}	0.016±0.0013^{c}	1.075±0.075^{b}	0.164±0.009^{e}	0.047±0.002^{d}
	Tmix	0.003±0.0002^{d}	0.016±0.0013^{c}	0.014±0.0009^{d}	0.839±0.058^{c}	0.084±0.006^{f}	0.030±0.003^{e}
	R^2	0.9125	0.9163	0.9264	0.9265	0.9541	0.9363
	P	***	***	***	***	***	***
After 4 days	wastewater	0.350±0.025^{a}	0.121±0.005^{a}	0.115±0.005^{a}	3.327±0.232^{a}	1.458±0.131^{a}	0.241±0.019^{a}
	T1	0.117±0.0035^{b}	0.051±0.0015^{b}	0.040±0.0001^{b}	0.841±0.042^{b}	0.236±0.007^{b}	0.098±0.058^{b}
	T2	0.069±0.0034^{c}	0.025±0.0012^{c}	0.020±0.0006^{d}	0.430±0.026^{c}	0.075±0.005^{d}	0.073±0.002^{c}
	T3	0.057±0.0017^{d}	0.021±0.0008^{c}	0.020±0.0002^{d}	0.338±0.027^{d}	0.090±0.004^{c}	0.067±0.003^{d}
	T4	0.052±0.0015^{d}	0.016±0.0006^{d}	0.030±0.0012^{c}	0.156±0.009^{e}	0.045±0.001^{f}	0.101±0.006^{b}
	T5	0.044±0.0022^{e}	0.010±0.0004^{d}	0.020±0.0008^{d}	0.143±0.006^{e}	0.059±0.002^{e}	0.057±0.003^{e}
	Tmix	0.032±0.0016^{f}	0.018±0.0013^{d}	0.020±0.0012^{d}	0.128±0.004^{e}	0.041±0.001^{f}	0.066±0.003^{d}
	R^2	0.9651	0.9427	0.9746	0.9743	0.9754	0.9336
	P	***	***	***	***	***	***

The same letters in each column indicate no significant differences at $p < 0.0001$ as analyzed by Duncan’s multiple-range test. Each Value is the average of 3 replicates ± SD.

and Pb (76%), while T2 had the highest Mn removal (95%) and T4 the best Fe removal (97%). Overall, Tmix consistently demonstrated superior heavy metal removal, with significant improvements over individual isolates.

Effect of Treated wastewater on the growth of *Vicia faba* (L.) seedlings

Results in Table 5 showed the positive impact of bacterial treatments on the growth of *Vicia faba* seedlings irrigated with wastewater. In comparison to untreated wastewater (positive control), bacterial treatments significantly improved several growth parameters, including shoot height, fresh and dry weights (both shoot and root), and leaf area. Among the treatments, the mixture of all five bacterial isolates (Tmix) had the most pronounced effects, suggesting a synergistic benefit from combining the bacterial strains. While the negative control (tap water) showed better growth than the positive control, bacterial treatments further enhanced growth, suggesting that the bacteria mitigate the inhibitory effects of wastewater. The Tmix treatment led to the highest values for most parameters, including shoot height (34.2 cm), shoot fresh weight (5.47 g), and root fresh weight (4.89 g), indicating that the bacteria promoted both shoot and root development, improving overall plant growth. T2 (sterilized wastewater treated with *Klebsiella sp*.) and T3 (sterilized wastewater treated with *Trabulsiella sp*.) also showed notable effects, particularly on root dry weight and leaf area.

Treated wastewater enhanced the photosynthetic efficiency and pigments of *Vicia faba* (L.) seedlings

Figure 3 demonstrates the impact of various bacterial treatments on the photosynthetic activity and pigment content of *Vicia faba* (L.) seedlings irrigated with sterilized treated wastewater. The data revealed significant improvements in photosynthetic efficiency (Fv/Fm), chlorophyll content (Chl a and Chl b), and carotenoid levels with **Stimulation of the antioxidative system of *Vicia faba* (L.) seedlings under treated wastewater**

Figure 4 shows that bacterial treatments significantly enhanced the activity of key antioxidant enzymes (catalase, superoxide dismutase, and peroxidase) in *Vicia faba* (L.) seedlings irrigated with sterilized treated wastewater. The results suggested that these bacterial inoculations, particularly sterilized wastewater treated with *Proteus sp.* (T1) and the mixed bacterial treatment (Tmix), help the plants manage oxidative stress by boosting their ability to detoxify reactive oxygen species (ROS). Increased catalase (CAT) and superoxide dismutase (SOD) activities indicate enhanced defense mechanisms against oxidative damage, while elevated peroxidase (POD) activity.

Effect of treated wastewater on the content of biochemical compounds in *Vicia faba* (L.) seedlings

Figure 5 provides detailed analysis of the effect of various bacterial treatments on several biochemical parameters in *Vicia faba* seedlings irrigated with sterilized treated wastewater.

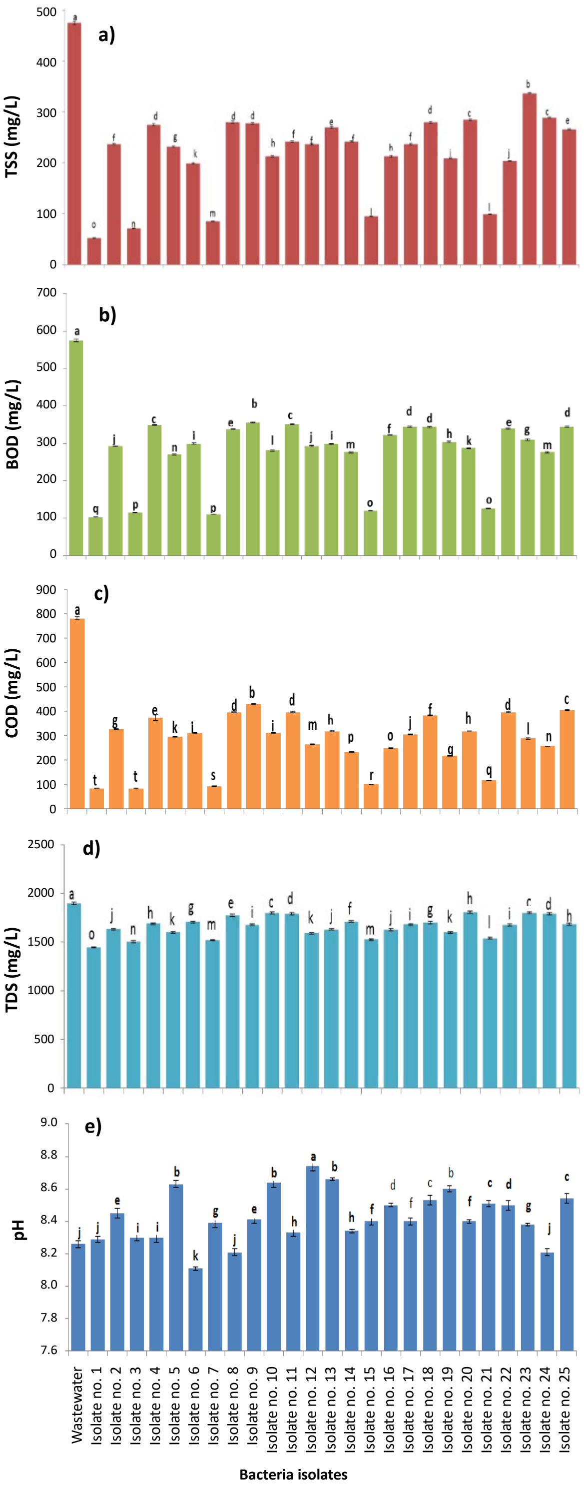


Figure 2. Estimation of the different parameters {(total suspended solids; TSS (a), biological oxygen demand; BOD (b), chemical oxygen demand; COD (c), total dissolved solids; TDS (d) and pH (e)} of wastewater and treated wastewater with different twenty-five bacterial isolates**.** Values are the means of 3 replicates ± standard error. Different small letters on different columns indicate significant differences between different treatments according to Duncan's multiple-range test (P ≤0.0001).

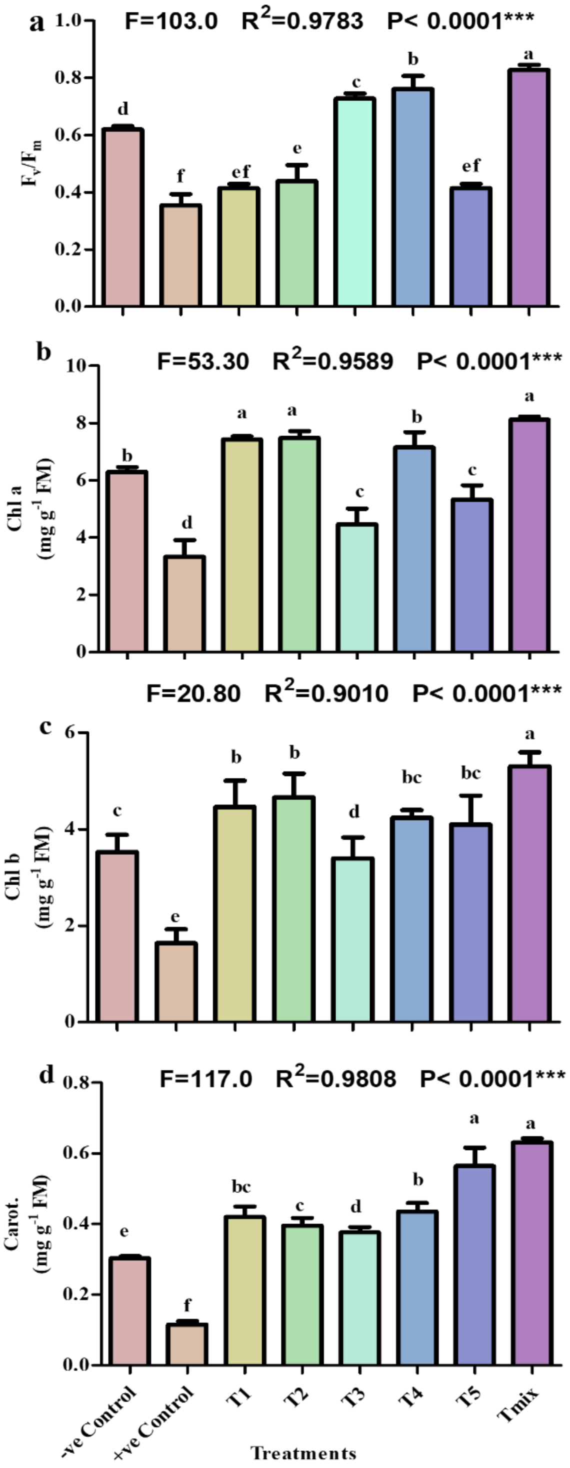


Figure 3. Effect of irrigation with wastewater (+ve control) and sterilized wastewater treated with *Proteus sp.* (T1), *Klebsiella sp.* (T2), *Trabulsiella sp.* (T3), *Escherichia sp.* (T4), *Kosakonia sp.* (T5), mixture of T1,T2,T3,T4,T5 (Tmix) on the photosynthetic activity (Fv/Fm) (a), Chl a (b), Chl b (c) and carotenoids (Carot.) (d) of 21-day old *Vicia faba* (L.) seedlings. Values are the means of 3 replicates ± standard error. Different small letters on different columns indicate significant differences between different treatments according to Duncan's multiple-range test (P ≤0.0001).

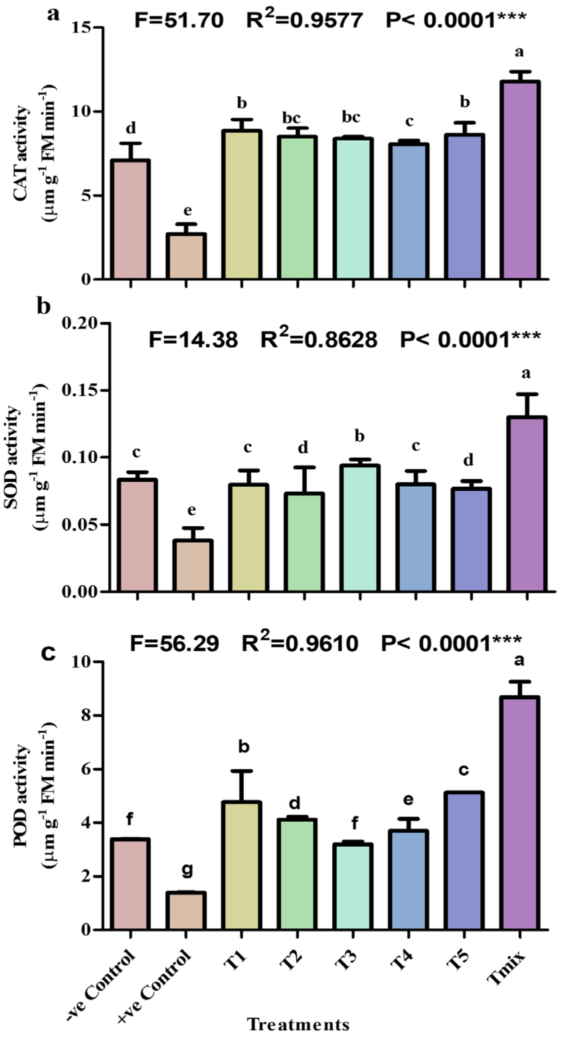


Figure 4. Effect of irrigation with wastewater (+ve control) and sterilized wastewater treated with *Proteus sp.* (T1), *Klebsiella sp.* (T2), *Trabulsiella sp.* (T3), *Escherichia sp.* (T4), *Kosakonia sp.* (T5), mixture of T1,T2,T3,T4,T5 (Tmix) on the activity of catalase enzyme (CAT) (a), superoxide dismutase enzyme (SOD) (b) and peroxidase enzyme (POD) (c) of 21-day old *Vicia faba* (L.) seedlings. Values are the means of 3 replicates ± standard error. Different small letters on different columns indicate significant differences between different treatments according to Duncan's multiple-range test (P ≤0.0001).

bacterial inoculation, particularly with sterilized wastewater treated with *Proteus sp.* (T1) and a mixture of all five bacterial isolates (Tmix). These treatments enhanced the plants' photosynthetic capacity, supported chlorophyll biosynthesis, and increased carotenoid content, which likely helped mitigate oxidative stress caused by wastewater irrigation. The combined bacterial treatment (Tmix) showed a synergistic effect, suggesting that mixed bacterial inoculations could offer broader benefits for plant growth.

The results show significant variations in the content of total soluble proteins (TSP), total soluble carbohydrates (TSC), total phenolics (T. Ph), total flavonoids (T. Flav), and ascorbic acid (A.A) across the different treatments, highlighting the potential role of bacterial inoculation in modulating plant metabolic responses under wastewater stress. The content of total soluble proteins was significantly higher in the positive control (+ve control), with a value of 54.63 mg/g DW, compared to the negative control (-ve control) and bacterial treatments. Among the bacterial treatments, sterilized wastewater treated with *Klebsiella sp.* (T2) and sterilized wastewater treated with *Trabulsiella sp.* (T3) exhibited higher TSP content (37.46 mg/g DW), suggesting that these bacterial isolates may enhance protein synthesis, potentially contributing to improved stress tolerance and overall plant health. The lower TSP levels in the negative control and some bacterial treatments indicate that while bacterial inoculation provides benefits, it does not always match the positive control's protein levels. The highest TSC content was observed in the positive control (+ve control) with 9.37 mg/g DW, significantly higher than the other treatments. Notably, the bacterial treatments, particularly sterilized wastewater treated with *Trabulsiella sp.* (T3) and sterilized wastewater treated with *Kosakonia sp.* (T5), showed moderate increases in TSC (6.43 mg/g DW and 6.42 mg/g DW, respectively), suggesting that these bacterial strains may aid in carbohydrate metabolism or enhance the plant's ability to store energy under stress conditions. This could be particularly beneficial for maintaining plant growth and physiological functions under the stress imposed by wastewater irrigation. Total phenolic content was significantly higher in the positive control (30.30 mg/g DW) compared to all bacterial treatments, with sterilized wastewater treated with *Kosakonia sp.* (T5) showing the highest phenolic content among the bacterial treatments (20.70 mg/g DW). Phenolics play a crucial role in plant defense and antioxidative properties, indicating that bacterial treatments, particularly sterilized wastewater treated with *Kosakonia sp.*, may help increase the plant's ability to cope with oxidative stress or microbial pathogens. The lower phenolic content in the negative control further emphasizes the beneficial effect of bacterial inoculation on secondary metabolite production. The total flavonoids followed a similar trend to the phenolic content, with the highest levels found in the positive control (9.63 mg/g DW). Among bacterial treatments, sterilized wastewater treated with *Kosakonia sp.* (T5) exhibited the highest flavonoid content (5.57 mg/g DW), which aligns with its ability to elevate other metabolic parameters like phenolics. Flavonoids are known for their role in protecting plants from oxidative damage, suggesting that sterilized wastewater treated with *Kosakonia sp.* could enhance the plant's defense mechanisms under wastewater irrigation. Ascorbic acid content was significantly higher in the mixed bacterial treatment (Tmix) at 37.13 mg/g FW, compared to all other treatments. This increase suggests that bacterial inoculation, particularly with a mixture of strains, can significantly enhance the plant's antioxidative capacity, which is crucial for mitigating oxidative stress caused by wastewater. The increase in ascorbic acid content supports the role of bacteria in boosting plant defense and health under stressful irrigation conditions. The significant increase in total soluble carbohydrates, phenolics, flavonoids, and ascorbic acid under bacterial treatments indicates that bacteria may enhance the plant's metabolic and antioxidant responses, improving stress resilience. While the positive control showed the highest values for most parameters, several bacterial treatments, particularly sterilized wastewater treated with *Kosakonia sp.* (T5) and the mixed treatment (Tmix), approached or even exceeded the positive control for certain metabolites, underscoring their potential as biostimulants for plants exposed to wastewater stress.

Table 4. The percentage removal efficiency of heavy metals (Cd, Cr, Cu, Fe, Mn and Pb) from sterilized wastewater treated with *Proteus sp.* (T1), *Klebsiella sp.* (T2), *Trabulsiella sp.* (T3), *Escherichia sp.* (T4), *Kosakonia sp*. (T5), and mixture of T1,T2,T3,T4,T5 (Tmix) after 2 and 4 days of their incubation

Days of Incubation	Water Samples	Removal efficiency of Cd (%)	Removal efficiency of Cr (%)	Removal efficiency of Cu (%)	Removal efficiency of Fe (%)	Removal efficiency of Mn (%)	Removal efficiency of Pb (%)
After 2 days	T1	17±0.51^{c}	30±0.29^{e}	22±0.34^{f}	73±0.63^{d}	40±0.52^{d}	31±0.41^{d}
	T2	17±0.17^{c}	67±0.43^{a}	63±0.41^{d}	90±0.57^{a}	64±0.36^{c}	54±0.52^{b}
	T3	33±0.99^{b}	37±0.29^{d}	29±0.28^{e}	74±0.90^{d}	41±0.43^{d}	44±0.42^{c}
	T4	33±0.66^{b}	53±0.58^{b}	71±0.99^{b}	83±0.99^{c}	87±0.27^{b}	37±0.41^{d}
	T5	17±1.02^{c}	53±0.61^{b}	69±0.89^{c}	83±1.21^{c}	86±0.42^{b}	50±0.51^{b}
	Tmix	50±0.76^{a}	47± 0.59^{c}	73±0.93^{a}	87±1.01^{b}	93±0.25^{a}	70±0.61^{a}
	R^2	0.9365	0.9985	0.9704	0.9435	0.9640	0.9378
	P	***	***	***	***	***	***
After 4 days	T1	67±0.54^{e}	58±0.68^{d}	69±0.97^{f}	75±0.77^{e}	84±0.61^{d}	59±0.51^{c}
	T2	80±0.92^{d}	79±0.79^{c}	81±1.05^{d}	87±1.55^{d}	95±0.52^{c}	70±0.71^{b}
	T3	84±1.02^{c}	83±0.86^{b}	85±1.27^{b}	90±1.70^{c}	94±0.82^{c}	72±0.72^{b}
	T4	85±1.05^{c}	87±0.98^{b}	78±1.09^{e}	95±1.85^{b}	97±1.1^{a}	58±0.53^{c}
	T5	87±1.00^{b}	92±1.13^{a}	83±1.55^{c}	96±1.88^{a}	96±1.4^{b}	76±0.82^{a}
	Tmix	91±1.10^{a}	85±1.01^{b}	87±1.78^{a}	96±1.92^{a}	97±13^{a}	73±0.91^{b}
	R^2	0.9347	0.9332	0.9557	0.9852	0.9520	0.9187
	P	***	***	***	***	***	***

The same letters in each column indicate no significant differences at $p < 0.0001$ as analyzed by Duncan's multiple-range test. Each Value is the average of 3 replicates ± SD.

Table 5. Effect of irrigation with wastewater (+ve control) and sterilized wastewater treated with *Proteus sp.* (T1), *Klebsiella sp.* (T2), *Trabulsiella sp.* (T3), *Escherichia sp.* (T4), *Kosakonia sp.* (T5), mixture of T1,T2,T3,T4,T5 (Tmix) on the growth parameters (shoot height, leaf area, shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) and root dry weight (RDW) of 21-day old *Vicia faba (*L.) seedlings

Treatments	Shoot height (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	Leaf area (cm^2)
-ve Control (Tap water)	30.60±0.53^{d}	3.133±0.04^{d}	3.936±0.03^{b}	2.638±0.04^{b}	2.033±0.04^{c}	26.36±0.65^{d}
+ve Control (wastewater)	18.40±0.43^{e}	2.033±0.04^{e}	2.106±0.04^{e}	1.343±0.02^{e}	0.866±0.04^{e}	17.00±0.31^{e}
T1	30.16±0.04^{d}	3.262±0.09^{c}	3.246±0.04^{d}	2.767±0.04^{b}	2.031±0.09^{c}	27.93±0.69^{c}
T2	30.80±1.25^{d}	3.301±0.08^{bc}	3.263±0.04^{d}	2.776±0.03^{b}	2.466±0.30^{b}	28.03±0.33^{b}
T3	32.60±0.45^{c}	3.462±0.06^{b}	3.366±0.04^{c}	2.466±0.06^{c}	1.566±0.82^{d}	28.43±0.33^{b}
T4	33.16±0.04^{b}	3.493±0.02^{b}	3.253±0.17^{d}	2.493±0.02^{c}	1.166±0.01^{d}	27.93±0.69^{c}
T5	32.16±0.81^{c}	3.135±0.21^{d}	3.509±0.14^{c}	2.377±0.04^{d}	2.166±0.04^{c}	28.03±0.33^{b}
Tmix	34.20±0.81^{a}	5.469±0.30^{a}	4.887±0.35^{a}	3.566±0.09^{a}	2.609±0.21^{a}	32.43±0.42^{a}
R^2	0.9797	0.9729	0.9565	0.9915	0.7574	0.9028
P	***	***	***	***	***	***

The same letters in each column indicate no significant differences at $p < 0.0001$ as analyzed by Duncan's multiple-range test. Each Value is the average of 3 replicates ± SD.

Enhancement of minerals of *Vicia faba* (L.) seedlings in response to treated wastewater

Figure 6 presents the effects of different bacterial suspensions on the mineral content (K, Ca, Mg, P, and N) of *Vicia faba* seedlings irrigated with sterilized treated wastewater. Data demonstrated significant variations in mineral uptake across the treatments, highlighting the influence of bacterial inoculation on enhancing nutrient absorption under wastewater irrigation. The highest potassium content was observed in the mixed bacterial treatment (Tmix) with 57.80 mg/g DW, significantly higher than all other treatments. Among the individual bacterial isolates, sterilized wastewater treated with *Klebsiella sp.* (T2) and sterilized wastewater treated with *Proteus sp.* (T1) also showed considerable increases in potassium levels (46.90 mg/g DW and 35.40 mg/g DW, respectively).

The positive control, which showed low potassium content (11.36 mg/g DW), further emphasizes the potential of bacterial treatments to enhance nutrient uptake compared to conventional irrigation. Sterilized wastewater treated with *Kosakonia sp.* (T5) and sterilized wastewater treated with *Klebsiella sp.* (T2) treated plants had the highest calcium content (27.36 mg/g DW and 26.95 mg/g DW, respectively), which were significantly higher than the negative control (14.43 mg/g DW) and the positive control (9.37 mg/g DW). The highest magnesium levels were found in the mixed bacterial treatment (Tmix) (9.13 mg/g DW), followed by sterilized wastewater treated with *Kosakonia sp.* (T5) (7.36 mg/g DW). Phosphorus content was highest in the mixed bacterial treatment (Tmix) with 7.86 mg/g DW, followed by sterilized wastewater treated with *Kosakonia sp.* (T5) (7.36 mg/g DW). The nitrogen content was also highest in the mixed bacterial treatment (Tmix) with 61.13 mg/g DW, significantly higher than all other treatments. Among individual bacterial isolates, sterilized wastewater treated with *Kosakonia sp.* (T5) showed the second-highest nitrogen content (50.36 mg/g DW).

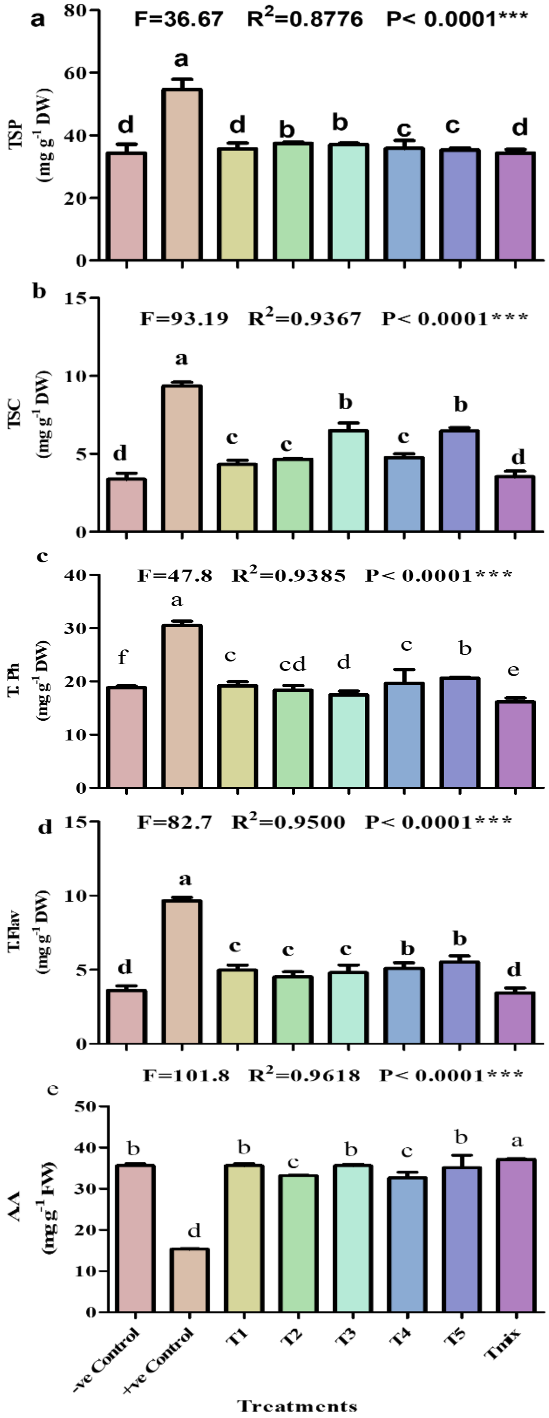


Figure 5. Effect of irrigation with wastewater (+ve control) and sterilized wastewater treated with *Proteus sp.* (T1), *Klebsiella sp.* (T2), *Trabulsiella sp.* (T3), *Escherichia sp.* (T4), *Kosakonia sp.* (T5), mixture of T1,T2,T3,T4,T5 (Tmix) on the content of total soluble proteins (TSP) (a) , total soluble carbohydrates (TSC) (b), total phenolics (T. Ph) (c), total flavonoids (T. Flav) (d) and ascorbic acid (A.A) (e) of 21-day old *Vicia faba* (L.) seedlings . Values are the means of 3 replicates ± standard error. Different small letters on different columns indicate significant differences between different treatments according to Duncan's multiple-range test ($P \leq 0.0001$).

DISCUSSION

This study investigated the potential of bacterial isolates from wastewater treatment plants to improve the quality of treated effluent, focusing on parameters such as total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), and PH.

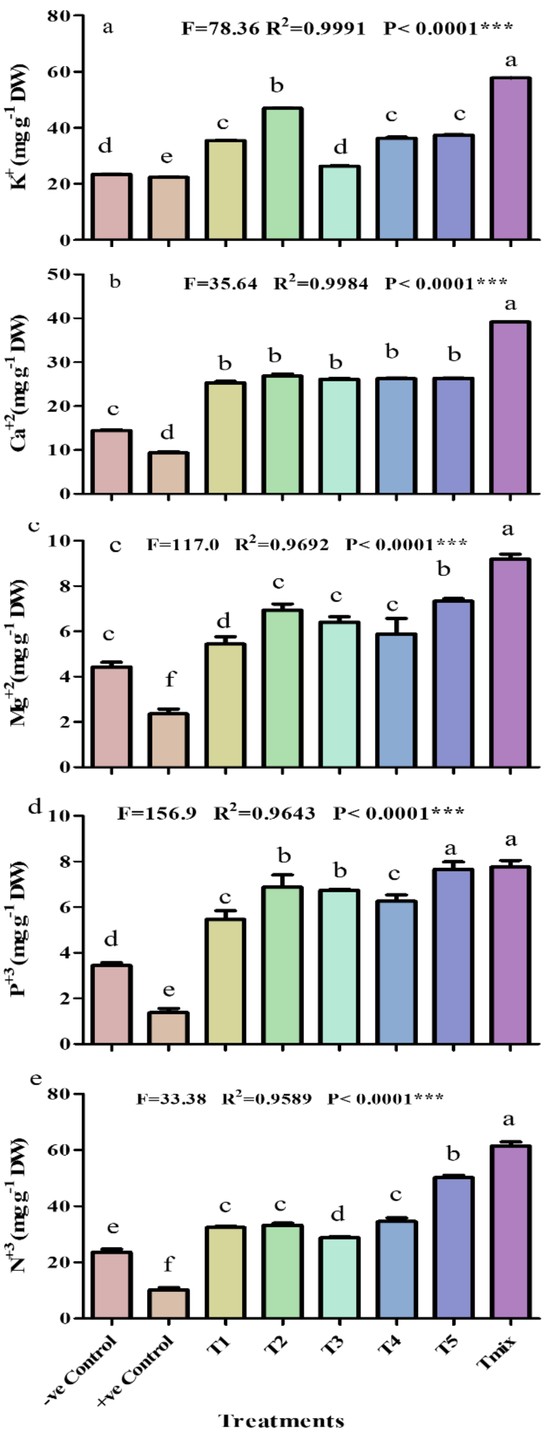


Figure 6. Effect of irrigation with wastewater (+ve control) and sterilized wastewater treated with *Proteus sp.* (T1), *Klebsiella sp.* (T2), *Trabulsiella sp.* (T3), *Escherichia sp.* (T4), *Kosakonia sp.* (T5), mixture of T1,T2,T3,T4,T5 (Tmix) on the content of minerals {(potassium; K (a), calcium; Ca (b), magnesium; Mg (c), phosphours; P (d) and nitrogen; N (e)} of 21-day old *Vicia faba* (L.) seedlings Values are means of 3 replicates ± standard error. Different small letters on different columns indicate significant differences between different treatments according to Duncan's multiple-range test (P ≤0.0001).

The bacteria were selected based on their morphological characteristics and subsequently tested for their ability to treat wastewater. The study also explored the use of bacterial consortia in wastewater treatment efficiency. The bacteria were tested for their ability to treat wastewater by measuring key parameters like TSS, BOD, COD, and TDS. Out of the twenty-five isolates, five demonstrated notable ability to treat wastewater, with their efficiency improving over the incubation period. These included isolates numbered 1, 3, 7, 15, and 21, which were further studied for their capacity to degrade pollutants. The identification of these isolates through 16S rRNA gene sequencing revealed their identity as *Proteus sp., Klebsiella sp., Trabulsiella sp., Escherichia sp.,* and *Kosakonia sp.,* all of which are known to have strong metabolic capabilities in degrading organic compounds and pollutants (Bastos *et al*., 2024).

The bacterial isolates were tested individually and as a consortium for their ability to treat Kafr El-Zayat wastewater after 2 and 4 days of incubation at 30°C. The treatment efficiency was measured based on efficiency in reductions of TSS, BOD, COD, TDS, and pH values. The results revealed that isolation no. 1 (presumed to be *Proteus sp*.) showed the highest efficiency in reducing all the parameters tested. After two days of incubation, isolation no. 1 achieved significant efficiency in TSS (60%), BOD (61%), COD (68%), and TDS (13%). While the reduction in TDS was relatively lower, the decrease in organic pollutants (BOD and COD) was considerable, indicating the bacterium's strong ability to degrade organic matter. The pH values, which remained slightly alkaline (ranging from 8.29 to 8.51), suggest that the bacteria might contribute to an increase in pH, potentially due to the degradation of organic acids during microbial activity (Song *et al*., 2018). After four days of incubation, the treatment efficiency improved further, with reductions of 35% in TSS, 31% in BOD, 26% in COD, and 1% in TDS. This increase in treatment efficiency over time indicates the progressive action of bacterial metabolism in breaking down pollutants. Notably, bacterial consortium treatment (combining isolates 1, 3, 7, 15 and 21) demonstrated superior performance compared to individual bacterial isolates. The consortium resulted in greater reductions in TSS (65%), BOD (68%), COD (70%), and TDS (10%), suggesting that a combination of bacterial strains could synergistically enhance pollutant removal (Li *et al*., 2023) .The positive control (activated sludge) also exhibited effective treatment, particularly in reducing BOD (78%) and COD (72%). However, the bacterial consortium generally outperformed positive control, particularly in reducing TSS and COD. These results support the hypothesis that bacterial consortia, consisting of complementary species, can more effectively treat wastewater than individual isolates (Jiang *et al*., 2021).

The treatment efficiency of individual bacterial isolates and their mixtures was compared with a negative control (untreated wastewater effluent) and a positive control (treated wastewater with activated sludge). The bacterial consortium's performance surpassed that of both controls, especially in terms of TSS and COD removal. After four days of incubation, the bacterial mixture showed reductions of 32% for TSS, 27% for BOD, 28% for COD, and 4% for TDS, indicating a significant improvement in wastewater quality. These results are in line with previous studies demonstrating the effectiveness of bacterial consortia in treating wastewater, as multiple bacterial strains may work synergistically to degrade a broader spectrum of pollutants (Lu *et al*., 2024). Also, the efficiency of bacterial isolates in reducing heavy metal concentrations in wastewater, demonstrating their potential as a bioremediation strategy. The treatment outcomes reveal significant improvements in metal removal efficiency with longer incubation periods, consistent with the metabolic activity and adaptation of bacteria over time. The use of a mixture of five bacterial isolates yielded the highest reductions across most metals, achieving up to 96% for Fe, 97% for Mn, and 91% for Cd after 4 days of incubation. This underscores the synergistic interactions among bacterial species, enhancing their collective capacity to uptake or transform heavy metals, as reported by Sarkar and Bhattacharjee (2025).

Among the individual isolates, sterilized wastewater treated with *Klebsiella sp.* and *Kosakonia sp*. demonstrated exceptional reductions for Fe and Mn, with efficiencies exceeding 90% after 4 days. These findings align with prior research that identifies these genera as potent heavy metal remediators due to their ability to produce extracellular polymeric substances (EPS), which enhance metal binding and sequestration (Chakdar *et al*., 2022). However, sterilized wastewater treated with Escherichia showed variability in its performance, achieving high removal efficiencies for Mn (97%) and Fe (95%) but relatively lower reductions for Pb (58%), indicating that its efficacy may be metal-specific or dependent on environmental conditions. Cadmium (Cd) exhibited consistently lower removal efficiencies compared to other metals, which may be attributed to its low bioavailability or strong complexation with organic matter in wastewater. This observation agrees with studies suggesting that Cd's ionic radius and chemical properties hinder its bioaccumulation by bacterial cells (Wang *et al*., 2024). Enhancing Cd removal could involve optimizing the pH, temperature, or adding chelating agents to increase its bioavailability. The superior performance of the bacterial consortium highlights the advantages of combining microbial isolates for bioremediation. The metabolic diversity and functional redundancy within the consortium likely contributed to the enhanced uptake and transformation of heavy metals, as each isolation may target different metals or deploy complementary mechanisms such as bioadsorption, enzymatic reduction, or intracellular accumulation (Rathour *et al.,* 2024). This finding supports the recommendation to prioritize mixed-culture approaches for complex wastewater treatment.

The ability of the bacterial isolates to treat wastewater can be attributed to their metabolic capabilities. Many of the bacteria identified in this study, including *Proteus sp., Klebsiella sp., Escherichia sp.,* and *Kosakonia sp.,* are well-known for their ability to degrade organic compounds and contribute to the bioremediation of polluted environments. These bacteria likely utilize the organic pollutants in the wastewater as a carbon and energy source, thereby reducing BOD and COD levels additionally, bacteria such as *Klebsiella sp.* and *Escherichia* sp. can reduce nitrogenous compounds through denitrification processes, further contributing to the overall improvement in water quality (Zhang *et al*., 2021).

The results of this study indicate that bacterial isolates and consortia have significant potential for enhancing the treatment of wastewater. The ability of these bacteria to reduce pollutants, particularly BOD, COD, and TSS, highlights their promise for use in wastewater treatment systems. The combination of microbial strains in a consortium could be particularly useful for treating complex wastewater mixtures, as it leverages the metabolic diversity of different bacterial species to degrade a wide range of pollutants. This approach could complement or even replace conventional biological treatment methods, which are often limited by the diversity and efficiency of the microbial populations they utilize (Kamboj and Choudhury, 2025).

The results of this study demonstrate that sterilized wastewater treated with bacteria significantly enhances the growth, photosynthetic efficiency, oxidative stress tolerance, and nutrient uptake of *Vicia faba* (L.) seedlings. The application of sterilized treated wastewater using bacterial isolates, particularly in combination (Tmix), improved various plant physiological parameters, suggesting the potential of using these bacteria for bioremediation and agricultural enhancement under wastewater irrigation (Saeed *et al*., 2022). Bacterial wastewater treatments, especially the mixture of five bacterial isolates (Tmix), showed a significant improvement in growth parameters, including shoot height, fresh weight, dry weight, and root development. These findings are consistent with previous research indicating that bacterial inoculation can promote plant growth by enhancing nutrient availability, mitigating oxidative stress, and improving the plant's overall physiological status (Begum *et al*., 2022). The Tmix treatment demonstrated a synergistic effect, with enhanced root and shoot development. This suggests that the combination of different bacterial species could offer broader benefits than single-strain treatments, possibly by targeting a wider range of pollutants or improving different aspects of plant health (Ram *et al*., 2022).

The significant improvements in photosynthetic efficiency (Fv/Fm), chlorophyll content (Chl a and Chl b), and carotenoid levels observed with bacterial treatments, particularly Tmix and *Proteus sp.* (T1), are noteworthy. Chlorophyll is essential for efficient photosynthesis, and carotenoids help protect the plant from oxidative damage, both of which were enhanced under bacterial treatments. The positive effects of bacterial inoculation on chlorophyll biosynthesis and photosynthetic activity may help mitigate the oxidative stress caused by pollutants in wastewater, thereby improving the overall health and productivity of plants (Jozay *et al*., 2024). The synergistic effect of the Tmix treatment suggests that bacterial interactions can lead to a more effective plant response to wastewater stress, reinforcing the potential of combined bacterial strains for agricultural bioremediation.

Wastewater treated with bacteria also significantly boosted the activity of key antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD), which are crucial for mitigating oxidative stress caused by the contaminants in wastewater. The elevated activities of these enzymes, particularly in plants treated with sterilized wastewater treated with *Proteus sp.* (T1) and sterilized wastewater treated with Tmix, indicate that the sterilized treated wastewater helped the plants detoxify reactive oxygen species (ROS) and protect cellular structures from oxidative damage (Sahu *et al*., 2022). The Tmix treatment showed the strongest synergistic effects, enhancing all three antioxidant enzyme activities. This suggests that sterilized treated wastewater can strengthen the plant's defense mechanisms against the damaging effects of wastewater irrigation, thus enhancing plant resilience under stress conditions.

The analysis of soluble proteins, carbohydrates, phenolics, flavonoids, and ascorbic acid (AA) revealed significant modulation of the plant's metabolic responses under bacterial treatments. For instance, sterilized wastewater treated with *Klebsiella sp.* (T2) and sterilized wastewater treated with *Trabulsiella sp*. (T3) increased the total soluble protein (TSP) content, which is vital for plant stress tolerance and growth. Similarly, the content of total soluble carbohydrates (TSC) was higher in bacterial treatments, especially with sterilized wastewater treated with *Trabulsiella sp*. (T3) and sterilized wastewater treated with *Kosakonia sp*. (T5), suggesting that Wastewater treated with bacteria may help plants better manage energy reserves and osmotic balance under stress conditions (Duan *et al.,* 2023). Sterilized wastewater treatment with mixture of bacteria (Tmix) enhanced ascorbic acid levels significantly, highlighting the ability of treated wastewater to boost antioxidant capacity, which is essential for mitigating oxidative stress (Mrozek *et al*., 2023). The increase in secondary metabolites, including phenolics and flavonoids, further supports the idea that sterilized treated wastewater enhances the plant's defensive responses and overall health. These findings are consistent with the well-documented role of phenolics and flavonoids in protecting plants from oxidative damage and microbial pathogens (Das *et al*., 2024).

The bacterial treatments also improved the uptake of essential minerals, including potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), and nitrogen (N). The mixed bacterial treatment (Tmix) showed the highest potassium and nitrogen contents, which are crucial for enzyme activation, osmotic regulation, and protein synthesis. These improvements in nutrient uptake are particularly important in wastewater-irrigated agriculture, where nutrient availability can often be limited or imbalanced. The enhanced mineral uptake, especially with sterilized treated wastewater with *Klebsiella sp.* (T2) and sterilized treated wastewater with *Kosakonia sp*. (T5), suggests that Wastewater treated with bacteria can not only mitigate the toxic effects of wastewater but also optimize nutrient availability for plant growth (Fallahi *et al*., 2021). This aligns with previous studies indicating that sterilized wastewater treated with bacteria can enhance nutrient mobilization in plants, improving their overall nutritional status (Singh *et al*., 2022b).

CONCLUSION

This study demonstrates that bacterial isolates from wastewater treatment plants have the potential to significantly improve wastewater quality, particularly in terms of reducing organic and suspended pollutants. The use of bacterial consortia offers an effective alternative or supplement to conventional wastewater treatment methods. Further research is needed to optimize bacterial consortium formulations and operational conditions for large-scale applications in wastewater treatment plants. Additionally, the molecular identification of the bacterial isolate provides valuable insight into the specific microbial communities involved in the bioremediation process and could guide future studies on the use of microbial-based technologies for sustainable wastewater management. The results of this study underscore the potential of using bacterial isolates, particularly in combination, for improving wastewater treatment and enhancing plant growth under wastewater stress. The sterilized wastewater treated with bacteria significantly improved plant growth, photosynthetic efficiency, antioxidant enzyme activity, and nutrient uptake in *Vicia faba (*L.) seedlings. The mixed bacterial treatment (Tmix) emerged as the most effective strategy, suggesting that synergistic effects among bacterial species can be harnessed for more efficient bioremediation and agricultural productivity. These findings provide valuable insights into the application of bioremediation strategies in wastewater-irrigated agriculture, particularly in areas with limited access to freshwater resources. Further research is needed to explore the mechanisms behind these effects and their practical applications in large-scale agricultural systems.

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