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Application of fertigation using biotreated agro-industrial wastewater (with *Bacillus paramycoides* MT477810) on the growth of two vegetables *Capsicum annuum* (var. Astra) and *Lycopersicon esculentum* (var. Kristina)

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ABSTRACT

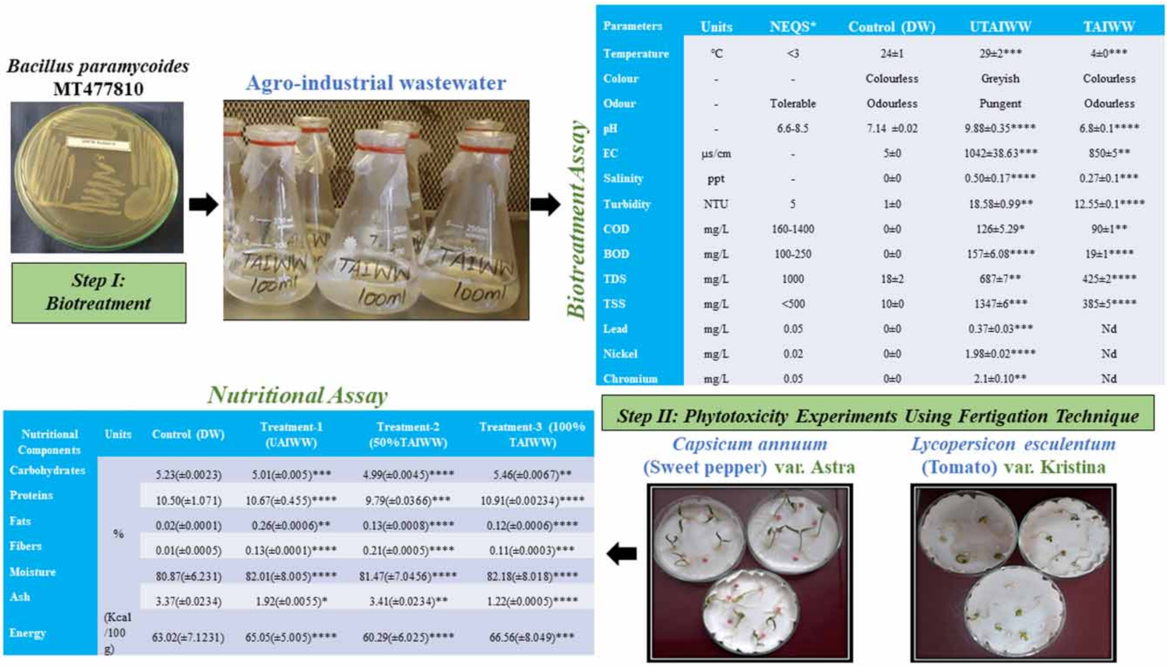
This research explored the use of the fertigation technique with treated agro-industrial wastewater to enhance the growth of *Capsicum annuum* (var. Astra) and *Lycopersicon esculentum* (var. Kristina). The wastewater, collected from Bajwa Agro Industries in Lahore, was biotreated using the bacterial strain *Bacillus paramycoides* MT477810, with a 10% inoculum incubated at 37 °C for 48 h. The bacterial strain achieved an 87% decolorization of the wastewater. Physicochemical analysis before and after biotreatment showed reductions in pH (60%), EC (22%), salinity (16%), turbidity (48%), COD (40%), BOD (73%), TDS (62%), and TSS (25%). The two vegetable varieties were fertigated with both biotreated and untreated wastewater to assess their effects on seed germination and plant growth. Results indicated a significant reduction in phytotoxicity (over 50%) in plants grown with biotreated wastewater. Additionally, plants irrigated with biotreated wastewater showed a 9–30% increase in fresh and dry weights compared to those irrigated with untreated wastewater. Nutritional analysis revealed that crops irrigated with treated wastewater had 12–48% higher nutrient content. These findings emphasize the effectiveness of biological wastewater treatment and fertigation in reducing pollutants and enhancing nutrient availability for vegetable crops.

Key words: agro-industrial wastewater, *Bacillus paramycoids*, biotreatment, fertigation, vegetables

HIGHLIGHTS

- Biotreated agro-industrial wastewater was applied via fertigation to grow *Capsicum annuum* (Astra) and *Lycopersicon esculentum* (Kristina).
- Biotreatment used *Bacillus paramycoides* MT477810 (10% inoculum) incubated at 37 °C for 48 h.
- The bacteria achieved up to 87% decolorization of wastewater.
- Irrigation with biotreated wastewater increased nutrient content by 12–48% and fresh/dry weight by 9–30%.

GRAPHICAL ABSTRACT



Parameters	Units	NEQS*	Control (DW)	UTAIWW	TAIWW
Temperature	°C	<3	24±1	29±2***	4±0***
Colour	-	-	Colourless	Greyish	Colourless
Odour	-	Tolerable	Odourless	Pungent	Odourless
pH	-	6.6-8.5	7.14 ±0.02	9.88±0.35****	6.8±0.1****
EC	µs/cm	-	5±0	1042±38.63***	850±5**
Salinity	ppt	-	0±0	0.50±0.17****	0.27±0.1***
Turbidity	NTU	5	1±0	18.58±0.99**	12.55±0.1****
COD	mg/L	160-1400	0±0	126±5.29*	90±1**
BOD	mg/L	100-250	0±0	157±6.08****	19±1****
TDS	mg/L	1000	18±2	687±7**	425±2****
TSS	mg/L	<500	10±0	1347±6***	385±5****
Lead	mg/L	0.05	0±0	0.37±0.03***	Nd
Nickel	mg/L	0.02	0±0	1.98±0.02****	Nd
Chromium	mg/L	0.05	0±0	2.1±0.10**	Nd

Nutritional Components	Units	Control (DW)	Treatment-1 (UAIWW)	Treatment-2 (50%TAIWW)	Treatment-3 (100% TAIWW)
Carbohydrates	%	5.23(±0.0023)	5.01(±0.005)***	4.99(±0.0045)****	5.46(±0.0067)**
Proteins		10.50(±1.071)	10.67(±0.455)****	9.79(±0.0366)***	10.91(±0.00234)****
Fats		0.02(±0.0001)	0.26(±0.0006)**	0.13(±0.0008)****	0.12(±0.0006)****
Fibers		0.01(±0.0005)	0.13(±0.0001)****	0.21(±0.0005)****	0.11(±0.0003)***
Moisture		80.87(±6.231)	82.01(±8.005)****	81.47(±7.0456)****	82.18(±8.018)****
Ash		3.37(±0.0234)	1.92(±0.0055)*	3.41(±0.0234)**	1.22(±0.0005)****
Energy	(Kcal /100 g)	63.02(±7.1231)	65.05(±5.005)****	60.29(±6.025)****	66.56(±8.049)***

1. INTRODUCTION

The leading causes of freshwater scarcity include rapid population growth, urbanization, industrialization, and excessive use of freshwater resources (Khoso *et al.* 2015; Qadir *et al.* 2023). Pakistan is currently ranked third among nations facing acute water scarcity (IMF 2015), with an annual per capita water availability of approximately 990 m^3 (Mancosu *et al.* 2015; World Bank 2022; WRI 2023). This scarcity is forcing farmers to repurpose untreated wastewater for agricultural irrigation (Khan *et al.* 2023), which is considered a significant alternative water source for agriculture due to its nutrient content (Fitton *et al.* 2019; Mahmood & Malik 2014). However, agro-industrial, domestic, and hospital effluents in Pakistan contain harmful substances such as amines, heavy metals (Chen *et al.* 2003, 2022; Cheriaa *et al.* 2012; Ibrahim *et al.* 2022), and additives like soaps, azo dyes, and surfactants (Azizullah *et al.* 2011, 2020), posing serious threats to biodiversity, including human health (Karem *et al.* 2021). Heavy metals, in particular, have indirect negative impacts on human health and phytotoxic effects on plants (Khawla *et al.* 2019a, 2019b; Al-Mutairi *et al.* 2023).

Microbe-mediated bioremediation is recognized as a crucial biological process due to its metabolic activities (Wojcieszynska *et al.* 2014; Sulistinah *et al.* 2019, 2023; Suyamud *et al.* 2020) in treating wastewater. Various types of wastewater, including hospital, domestic, textile (Suksaroj *et al.* 2021), pharmaceutical, and industrial wastewater (Ogugbue & Sawidis 2011a; Dwivedi & Tomar 2023), can be treated using bacteria. These microorganisms can transform harmful contaminants into non-toxic molecules due to their biological activity and catabolic adaptability (Mahmood *et al.* 2011a, 2011b; Syed & Chinthala 2022). Most local indigenous bacterial isolates can be employed to degrade colour and detoxify wastewater sources (Phugare *et al.* 2011c). Recent research highlights the capacity of these native bacterial strains to efficiently break down a range of synthetic dyes, emphasizing their economic and environmental benefits (Ali *et al.* 2022). Advances in metagenomic approaches have improved our understanding of these species' roles in bioremediation by identifying important bacterial species and their metabolic pathways (Sharma & Kumar 2023). Furthermore, combining physicochemical techniques with bacterial consortia has shown promising results in enhancing the overall efficiency of wastewater treatment processes (Gupta *et al.* 2021).

Bioremediation using developed biotreatment techniques offers a low-tech, practical, and affordable solution for developing countries such as Pakistan (Adel *et al.* 2024). Many chemical compounds present in wastewater can be readily biodegraded and detoxified (Shomar *et al.* 2020a, 2020b; Kong *et al.* 2024). Removing pollutants and contaminants from the environment is essential for achieving sustainable progress and positive environmental impact (Mora-Ravelo *et al.* 2017a, 2017b), which largely depends on biotic techniques such as the biotreatment of agro-industrial wastewater for use as irrigation water (Kostka *et al.* 2011a, 2011b; Modi *et al.* 2022; Baawain *et al.* 2023).

For this purpose, fertigation is a cutting-edge agricultural method that combines irrigation and fertilization, allowing precise application of water and nutrients directly to the plant root zone (Chen *et al.* 2020). This technique is highly beneficial in modern agriculture as it maximizes water use, reduces nutrient losses, and improves nutrient uptake efficiency (Simsek & Sarihan 2016). Using treated agro-industrial wastewater (TAIWW) in fertigation systems addresses water scarcity and nutrient management in agriculture sustainably (Chen *et al.* 2020). Treated wastewater not only provides a valuable source of nutrients but also offers additional benefits. According to Mekki *et al.* (2013), in fertigation, the amount of nutrients supplied is calculated based on the reference chemical element, which is the nutrient required by the crop in the smallest amount within the applied wastewater. This method ensures that crops receive precisely the right amount of nutrients, reducing the risk of over-fertilization and its environmental impacts, such as nutrient runoff and groundwater contamination (Zhang *et al.* 2019a, 2019b).

These practices are typically carried out in sterile laboratory settings or as *ex-situ* prototypes (Ogugbue & Sawidis 2011b; Sanders 2023). This approach could serve as a reliable alternative source to meet the growing demand for groundwater/freshwater for crop irrigation (Chung *et al.* 2023). The use of bacterial-treated agro-industrial wastewater in fertigation systems offers a sustainable and efficient solution for crop irrigation (García-Gómez *et al.* 2019). As the demand for food production continues to rise, integrating treated wastewater into fertigation systems will play an increasingly important role in ensuring the sustainability and resilience of agricultural systems worldwide (Simsek & Sarihan 2016).

With this in mind, the present study targeted the following objectives: (1) characterization of the agro-industrial wastewater under study, (2) investigation of the potential of *Bacillus paramycoides* for degrading pollutants in agro-industrial wastewater, and (3) application of fertigation to compare the effects of raw and biotreated agro-industrial wastewater on the seed germination and growth of two vegetables, *C. annuum* var. Astra and *L. esculentum* var. Kristina.

2. MATERIALS AND METHODS

2.1. Collection and analysis of wastewater

The experiment was conducted in the Plant Biotechnology Laboratory, Department of Botany, GC University Lahore. A total of 100 L of wastewater was collected in sterilized bottles from the main outflow point of Bajwa Agro-Industry, Lahore, Pakistan (N 31°35′11.3208, E 74°22′38.6652) in triplicates, based on its distance from the point of discharge. The wastewater was filtered in the laboratory and kept at room temperature for physicochemical analysis. The samples were analysed immediately to characterize them, ensuring bacterial viability and preventing the organic substances from degrading naturally. The physicochemical parameters – colour, odour, temperature, pH, electrical conductivity (EC), total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), biological oxygen demand (BOD_5), salinity (ppt), and turbidity (NTU) – were all examined according to the American Public Health Association (APHA) (2005) guidelines using a portable photometer (HI-97727), digital thermometer (HUBDIC), pH meter (HQ30D pH, EC, and DO meter), portable EC meter (HQ30D pH, EC, and DO meter), COD digester, BOD digester, and turbidity meter, respectively (Rashid *et al.* 2020). Using an atomic absorption spectrophotometer (AA 7000 F with Autosampler and Hydride Vapour Generator, Shimadzu, Japan), the concentrations of the following heavy metals were measured: lead (Pb), nickel (Ni), and chromium (Cr) (Rashid *et al.* 2020). The physicochemical parameters were tested twice, before and after biotreatment, and the results were compared to the National Environmental Quality Standards (NEQS).

2.2. Decolourization experiment

2.2.1. Bacterial culture maintenance

The decolourization experiment involved the use of *Bacillus paramycoids* MT477810, a bacterial isolate with over 70% decolourization capacity (Rashid *et al.* 2020), to evaluate the bioremediation potential of agro-industrial wastewater samples. *B. paramycoides* was selected for wastewater treatment due to its robust ability to degrade complex organic pollutants and its high tolerance to varying environmental conditions, such as pH and temperature. This bacterial strain is renowned for its enzymatic activity, which efficiently breaks down organic matter, thereby reducing BOD and COD levels in wastewater (Li *et al.* 2018; Zhang *et al.* 2019a, 2019b). Additionally, it has demonstrated effective nutrient cycling, making it ideal for bioremediation applications where nutrient recovery is crucial, such as in agricultural wastewater treatment, where the goal is to reuse treated water for irrigation (Patel & Vashi 2010a, 2010b; Rashid *et al.* 2020). The bacterial isolate was cultured on nutrient agar plates and purified by streaking (Liu *et al.* 2017). The purified bacterial culture was then transferred to Luria-Bertani medium slants (Liao *et al.* 2013) and stored in a refrigerator (Eyler 2013).

2.2.2. Treatment of wastewater

The agro-industrial wastewater was treated with the bacterial isolate *Bacillus paramycoids* MT477810. The wastewater was inoculated with 10% inoculum, containing approximately 1.5×10^8 CFU/mL of bacterial mass, and incubated at 37 °C for 48 h (Madukasi *et al.* 2010; Rashid *et al.* 2020). During this time, the biomass of the bacterial cells increased by nearly 1.6 times, indicating substantial cell growth. This growth facilitated more effective biodegradation of toxins, helping to reduce contaminant levels. After 48 h, the bacterial population began to stabilize as nutrition availability decreased. The decolourization activity was then assessed using a spectrophotometer. The treated wastewater was transferred from flasks into autoclaved Falcon tubes and stored in a refrigerator for future use (Mignard & Flandrois 2006) (Figure 1).

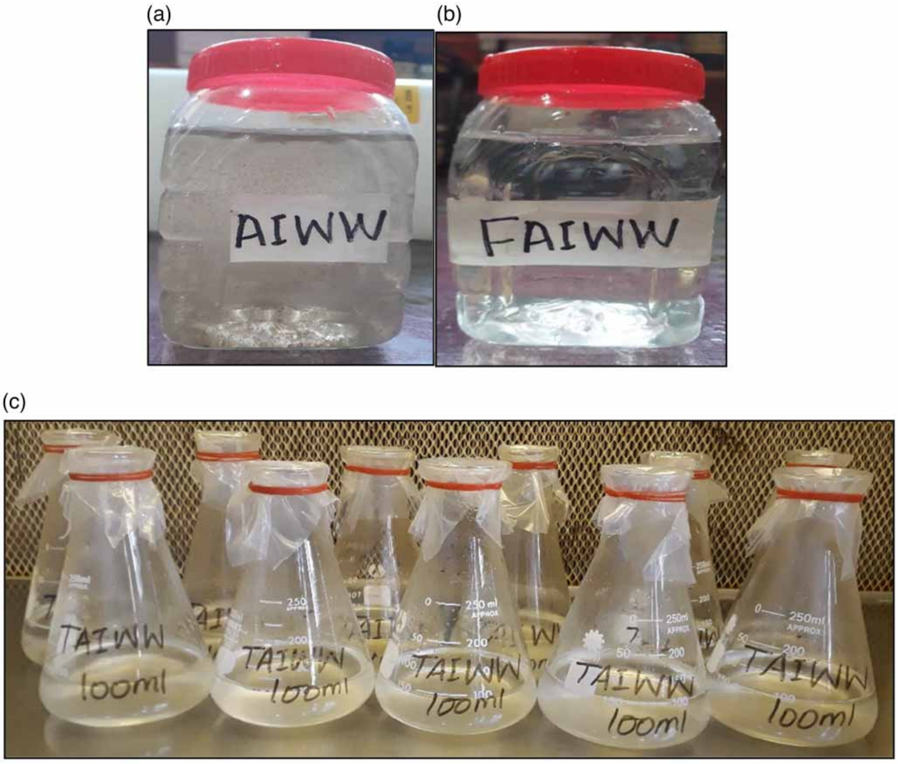


Figure 1 | Different wastewater samples (a) raw agro-industrial wastewater, (b) filtered agro-industrial wastewater, and (c) treated agro-industrial wastewater.

2.3. Seed procurement and germination

Seeds of two vegetables, i.e., *C. annuum* var. Astra and *L. esculentum* var. Kristina, were procured from the Federal Seed Certification Department in Lahore, Pakistan. The seeds were germinated to assess the appropriateness of UTAIWW and TAIWW (Khalil & Kakar 2011; Aslam *et al.* 2023). Each batch of seeds was surface-sterilized using a 70% ethanol solution followed by a 30% bleach solution under aseptic conditions (Rashid *et al.* 2021). Sterilized seeds (five per batch) were then placed in sterilized Petri dishes, containing a double layer of filter paper. The plates were incubated using a completely randomized design in a growth room under optimized conditions at 26 °C ± 1, with a light intensity of 100–150 μmol/m^2 s and 18/6-h light/dark cycle, considered optimal for vegetative growth (Kang *et al.* 2013). Initial seed germination was observed on the 4th day, with the final observation was on the 10th day. Seeding growth was monitored for 21 days to measure growth parameters such as seeding length and fresh and dry weights.

2.4. Pot experiment using fertigation technique

Fifteen-day-old seedlings of *C. annuum* and *L. esculentum* were transplanted into medium-sized pots (15 × 15 cm) at a temperature of 26 °C ± 1, using garden soil procured from the Botanic Garden of GC University, Lahore. The seedlings were grown in four sets of pots, with five seedlings in each pot, and irrigated using the following fertigation treatments: the first set received autoclaved distilled water (DW), the second set received raw, untreated agro-industrial wastewater (UTAIWW), the third set received a 50% dilution of the wastewater, and the fourth set received 8 weeks of irrigation.

Seed germination was monitored daily, with seeds considered germinated when sprouts became apparent and measurable. The weight and length of the seedlings were recorded daily. A seed was deemed germinated if its root was discernible and measurable (i.e., more than 0.5 mm). For each experimental set, the length of the root and shoot of the germinated seedlings was measured. The shoot length was measured from the base of the primary leaf to the hypocotyl base, while the root length was measured from the root tip to the hypocotyl base, both in centimetres (cm). The total seedling length, also in cm, was calculated by summing the lengths of the shoot and root. The dry weights of the roots and shoots were determined after oven-drying them overnight at 65 °C.

2.5. Plant nutritional analysis

The nutritional analyses of both vegetables, grown in different water treatments, were carried out in the Meat and Dairy Lab at the Food and Biotechnology Research Centre (FBRC), Pakistan Council of Scientific and Industrial Research (PCSIR), Lahore. Nutritional compositions were determined using the following standard methods in both vegetables.

2.5.1. Moisture content

An initial sample of 2 g was taken in a Petri dish and placed in a drying oven for 4 h. The sample was then removed from the oven and transferred to a desiccating unit, and the dried sample was weighed accurately (Singh *et al.* 2001). Equation (1) was used to determine the percentage of moisture content.

$$\%\text{Moisture} = (\text{Loss in weight}/\text{Weight of sample}) \times 100 \tag{1}$$

2.5.2. Ash content

Ash is the measurement of the mineral content. A 10 g sample was taken in a crucible, and the initial weight was noted. The crucible was placed in a cold muffle furnace for 24 h and brought up gradually to remove moisture. The ignition point of the furnace was 525 °C. After 2 days, the crucible was transferred to the desiccating unit for 2–3 min, and the dried weight of the sample was recorded (Varnavskaya *et al.* 2023). Equation (2) was used to determine the ash percentage.

$$\%\text{Ash} = (\text{Weight of ash}/\text{Weight of sample}) \times 100 \tag{2}$$

2.5.3. Protein estimation

Proteins were estimated using Kjeldahl's method. Each vegetable sample (1 g) was taken into a digestion flask, and an equal amount of digestion mixture (K_2SO_4, $CuSO_4$, and SeO_2), and 20 mL H_2SO_4 were added. Digestion flasks were heated until a green colour was attained. After cooling, the digested samples were transferred to a volumetric flask, and the volume was raised to 100 mL with DW. Boric acid (2%) was taken in a 100 mL beaker and connected with the tip of the condenser of distillate. Five to ten millilitres of the digested sample was pipetted into the distillation flask. After adding sodium hydroxide solution, distillation continued until the colour of the boric acid solution changed. The collected distillate was titrated against standard HCl solutions (Gupta *et al.* 2005). Equations (3) and (4) were used to determine the percentage of proteins in each vegetable sample.

$$\%\text{Nitrogen} = \left[\begin{array}{l}(\text{Titre} \times \text{Total volume made} \times \text{Normality of acid} \times 14)\\ /(\text{Volumes taken} \times \text{Weight of sample} \times 1,000)\end{array}\right] \times 100 \tag{3}$$

$$\%\text{Protein} = \%\text{Nitrogen} \times \text{Factor } (6.25) \tag{4}$$

2.5.4. Fat estimation

The Soxhlet method was used for fat estimation, taking a 4 g sample of the vegetables. All samples were dried in aluminium dishes in a drying oven for 2 h at 125 °C. The dried samples were then placed into a Soxhlet extraction thimble. Fat was extracted from each sample using hexane, condensing at 6 to 6 drops per second in the Soxhlet extraction unit for 5 h. After extraction, all samples were placed in an oven, and the dried weight was recorded (Hewavitharana *et al.* 2020). The percentage of fats was determined using Equation (5):

$$\%\text{Fat} = (\text{Weight of fat}/\text{Weight of sample}) \times 100 \tag{5}$$

2.5.5. Crude fibre content

The fibre content was estimated by the method reported by Pisarikova *et al.* (2007) with a few modifications. A fat-free sample (2 g) was taken in a 500 mL beaker and boiled constantly for 30 min in 200 mL of 1.25% H_2SO_4; DW was added at frequent intervals to maintain a constant volume in the flask. It was then filtered using muslin cloth, and the acid was completely removed by washing the residue with hot water. The residue was transferred to a beaker and subjected to 30 min boiling with 200 mL 1.25% NaOH, then filtered and washed with hot water to make it alkali-free and with alcohol and diethyl ether. It was then transferred into a crucible and dried overnight at 105 °C. The ash was prepared by placing the crucible in a muffle furnace at 550 °C for 5 h, and it was cooled and weighed. The crude fibre content was calculated using Equation (6):

$$\%\text{Crude fibre} = [(\text{Crucible weight with ash} - \text{Crucible weight})/\text{Weight of sample}] \times 100 \tag{6}$$

2.5.6. Carbohydrate content

The percentage of carbohydrate content was calculated using Equation (7) (FAO 2013):

$$\%\text{Carbohydrate} = [100-(\%\text{Moisture} + \%\text{Ash} + \%\text{Proteins} + \%\text{Fat} + \%\text{Fibre})] \tag{7}$$

2.6. Statistical analysis

The data were presented as means ± standard deviation (SD). Statistical analysis was performed using a *t*-test with Welch's correction via GraphPad Prism software® 2020. Two-tailed *p*-value calculations were made for each of the parameters (pH, EC, TSS, TDS, COD, BOD_5, salinity, and turbidity), with three replicate values for each parameter. Results were compared before and after biotreatment, with differences considered significant when the probability threshold was less than 0.05. The significance levels are represented as follows: $^{*}p < 0.1$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Each seed experiment was statistically analysed using one-way ANOVA, with the indices serving as the effects and the concentrations of biotreated agro-industrial wastewater as the factors.

3. RESULTS

3.1. Characterization of agro-industrial wastewater

The NEQS (National Environment Quality Standards 2000) were used to compare the physiochemical properties of DW, UTAIWW, and TAIWW, as shown in Table 1. According to the data, UTAIWW exhibited significantly higher values for most parameters, indicating poor water quality. These parameters include salinity, turbidity, EC, COD, BOD, TSS, and TDS. TAIWW significantly reduced these values, improving water quality, though it did not fully reach the baseline of DW. Specially, pH was reduced by 60.17%, EC improved by 22%, salinity by 16%, turbidity by 48%, COD by 40%, BOD by 73%, TDS by 62%, and TSS by 25%. Additionally, three heavy metals (lead, nickel, and chromium) were detected in the untreated wastewater but remained undetected

Table 1 | Physiochemical characterization of different treatments

Parameters	Units	NEQS*	Control (DW)	UTAIWW	TAIWW
Temperature	°C	<3	24 ± 1	29 ± 2***	4 ± 0***
Colour	–	–	Colourless	Greyish	Colourless
Odour	–	Tolerable	Odourless	Pungent	Odourless
pH	–	6.6–8.5	7.14 ± 0.02	9.88 ± 0.35****	6.8 ± 0.1****
EC	µs/cm	–	5 ± 0	1,042 ± 38.63***	850 ± 5**
Salinity	ppt	–	0 ± 0	0.50 ± 0.17****	0.27 ± 0.1***
Turbidity	NTU	5	1 ± 0	18.58 ± 0.99**	12.55 ± 0.1****
COD	mg/L	160–1,400	0 ± 0	126 ± 5.29*	90 ± 1**
BOD	mg/L	100–250	0 ± 0	157 ± 6.08****	19 ± 1****
TDS	mg/L	1,000	18 ± 2	687 ± 7**	425 ± 2****
TSS	mg/L	<500	10 ± 0	1,347 ± 6***	385 ± 5*****
Lead	mg/L	0.05	0 ± 0	0.37 ± 0.03***	Nd
Nickel	mg/L	0.02	0 ± 0	1.98 ± 0.02****	Nd
Chromium	mg/L	0.05	0 ± 0	2.1 ± 0.10**	Nd

The significance levels are indicated as follows: $*p < 0.1$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. Results are presented as the mean ± standard deviation of triplicate measurements.

DW, distilled water; UTAIWW, untreated agro-industrial wastewater; TAIWW, treated agro-industrial wastewater; NEQS, National Environment Quality Standards; Nd, not detected.

after treatment. The differences in pH, EC, and other parameters were statistically significant, with *p*-values ranging from $*p < 0.1$ to $****p < 0.0001$. These findings highlight the effectiveness of the treatment process in improving water quality.

3.2. Germination experiment using fertigation technique

The experiment was conducted using the two selected vegetables, i.e., *L. esculentum* and *C. annuum*, which were grown in medium-sized pots to compare the effects of biotreated and UTAIWW.

3.2.1. Germination parameters

C. annuum and *L. esculentum* seeds were allowed to germinate and grow for a total of 21 days. At the end of this period, germination data were collected and analysed. Among the different wastewater treatments, the biotreated wastewater resulted in significantly higher germination parameters compared to the control (Tables 2 and 3).

Table 2 | Germination parameters of *C. annuum* under different treatments

Germination parameters	Units	Control (DW)	Treatment-1 (UTAIWW)	Treatment-2 (50% TAIWW)	Treatment-3 (100% TAIWW)
Final germination percentage	%	82.22(± 3.8451, 2.22)	91.10(±3.8502, 2.223)***	84.33(±3.7535, 2.167)***	97.77(±3.8502, 2.223)****
Germination energy	%	4.44(± 3.8451, 2.22)	13.33(±6.67, 3.8509)****	4.44(±3.8451, 2.22)**	26.66(±6.665, 3.8480)****
Germination index (GI)	%	391.52(± 18.3077, 10.57)	433.83(±18.3366, 10.5866)****	402.09(±18.3077, 10.57)****	444.42(±31.77, 18.3424)***
Germination velocity (GVe)	cm	429.30(± 18.608, 10.7433)	464.42(±21.1483, 12.21)*	444.42(±19.2459, 11.1116)***	763.84(±16.5930, 9.58)****
Germination vigour index (GVI)	cm	6.59(± 0.1501, 0.0866)	6.67(±0.2500, 0.1443)****	6.84(±0.1385, 0.08)****	7.15(±0.1963, 0.1133)**
Mean germination time (MGT)	s	82.22(± 3.8451, 2.22)	91.10(±3.8502, 2.223)***	84.33(±3.7535, 2.167)****	97.77(±3.8502, 2.223)**

The value outside the parentheses represents the mean, while the values in parentheses represent the standard deviation and standard error; the significance levels are indicated as follows: $*p < 0.1$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

DW, distilled water; UTAIWW, untreated agro-industrial wastewater; TAIWW, treated agro-industrial wastewater.

Table 3 | Germination parameters of *L. esculentum* under different treatments

Germination parameters	Units	Control (DW)	Treatment-1 (UTAIWW)	Treatment-2 (50% TAIWW)	Treatment-3 (100% TAIWW)
Final germination percentage	%	82.22(± 7.6960, 4.4433)	91.11(±3.8510, 2.2233) ****	79.99(±6.6650, 3.8480) ****	97.78(±3.8510, 2.2233) ****
Germination energy	%	11.11(± 3.8451, 2.22)	11.11(±7.6960, 4.4433) *	8.89(±3.8451, 2.22)****	17.78(±3.8509, 2.2233) ****
Germination velocity	%	391.51(± 36.6502, 21.16)	433.84(±18.3308, 10.5833)****	380.94(±31.74, 18.3251) **	465.6(±18.3424, 10.59) ****
Germination index	cm	1356.58(± 126.9892)	1396.67(±59.0340, 34.0833)***	1044.47(±248.6035, 143.5313)****	2023.98(±79.7148, 46.0233)****
Germination vigour index	cm	6.14(± 0.1213, 0.07)	6.21(±0.0520, 0.03) ****	6.17(±0.0923, 0.0533)***	6.30(±0.0173, 0.01) ****
Mean germination time	s	82.22(± 7.6960, 4.4433)	91.11(±3.8510, 2.2233) **	79.99(±6.6650, 3.8480) ***	97.78(±3.8510, 2.2233) ****

The value outside the parentheses represents the mean, while the values in parentheses represent the standard deviation and standard error. The significance levels are indicated as follows: *$p < 0.1$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

DW, distilled water; UTAIWW, untreated agro-industrial wastewater; TAIWW, treated agro-industrial wastewater.

The seed germination percentage for both vegetables grown in different concentrations of TAIWW and UTAIWW was within the range of 80–100%. Seeds of both vegetables grown in UTAIWW sprouted between 80 and 90%. Seeds of both vegetables grown in TAIWW sprouted between 95 and 100% (Figure 2).

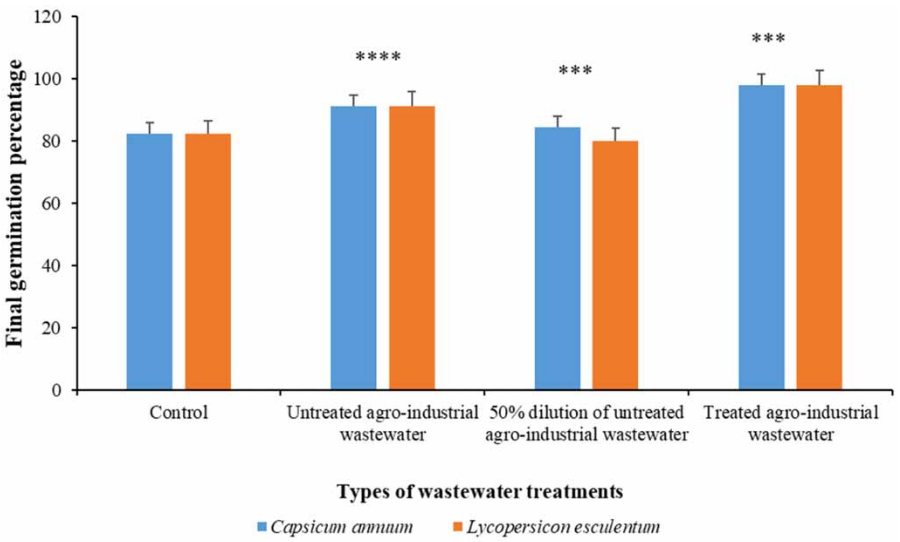


Figure 2 | Final germination percentage (FGP) of *C. annuum* and *L. esculentum* under different wastewater treatments.

The germination energy for both vegetables grown in different concentrations of TAIWW and UTAIWW was within the range of 4–30%. Seeds of both vegetables grown in UTAIWW sprouted between 10 and 15%. Seeds of both vegetables grown in TAIWW sprouted between 15 and 30% (Figure 3).

The germination vigour index for *C. annuum* grown in untreated wastewater was 6.67 cm, and the GVI for *C. annuum* seeds grown in TAIWW was 7.15 cm. Likewise, the GVI for *L. esculentum* seeds grown in untreated wastewater was 6.21 cm, and the GVI for *L. esculentum* seeds grown in TAIWW was 6.30 cm (Figure 4).

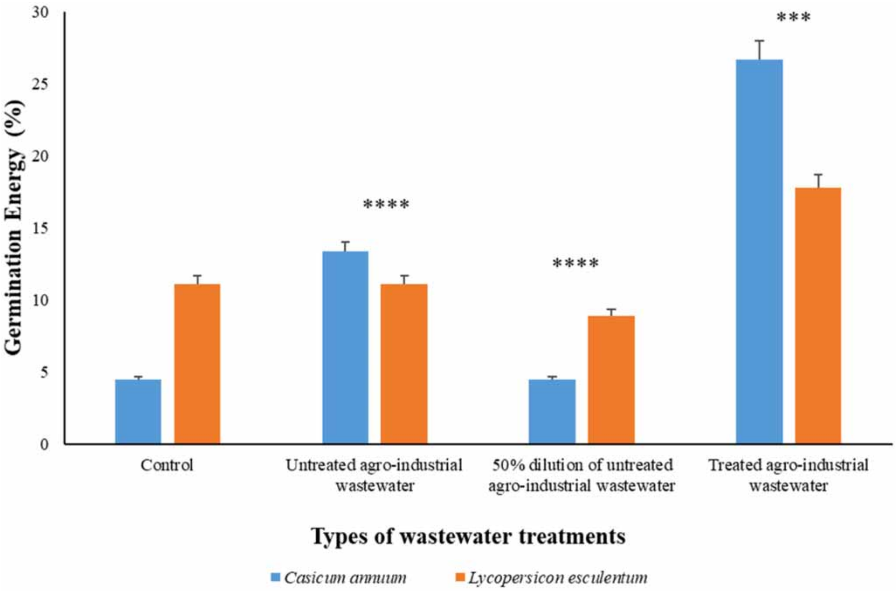


Figure 3 | Germination energy (GE) of *C. annuum* and *L. esculentum* under different wastewater treatments.

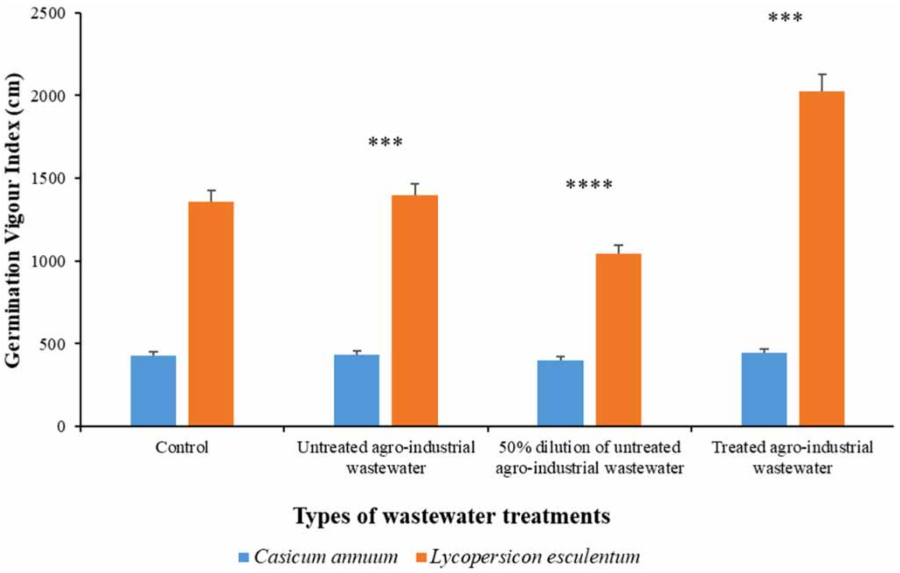


Figure 4 | Germination vigour index (GVI) of *C. annuum* and *L. esculentum* under different wastewater treatments.

The germination meantime for *C. annuum* grown in untreated wastewater was 91.11 s, and the GVI for *C. annuum* seeds grown in TAIWW was 97.77 s. Likewise, the GVI for *L. esculentum* seeds grown in untreated wastewater was 91.10 s, and the GVI for *L. esculentum* seeds grown in TAIWW was 97.78 s (Figure 5).

3.2.2. Measurement of morphological parameters

Various morphological parameters were observed under different types of water. Distinct differences in morphological parameters were evident between the different water treatments and controls. The morphological parameters supported by biotreated wastewater showed the most significant improvements.

3.2.3. Morphological parameters observed for *C. annuum*

The lengths of *C. annuum* seedlings (shoot and root lengths) were longer in TAIWW concentrations than in UTAIWW concentrations. The weights of the *C. annuum* roots and shoots were also higher in treated wastewater concentrations than in untreated wastewater concentrations (Table 4).

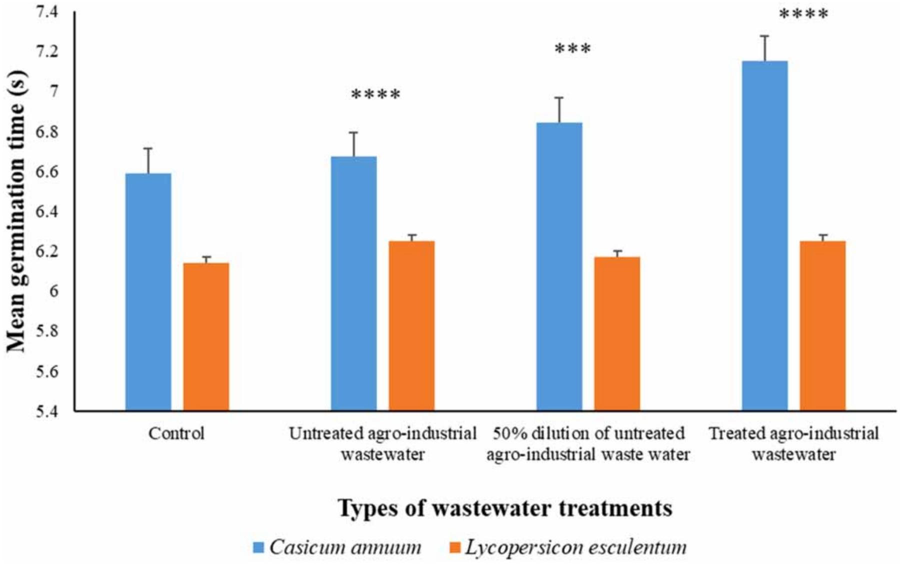


Figure 5 | Mean germination time (MGT) of *C. annuum* and *L. esculentum* under different wastewater treatments.

Table 4 | Morphological parameters of *C. annuum* under different treatments

Morphological parameters	Units	Control (DW)	Treatment-1 (UTAIWW)	Treatment-2 (50% TAIWW)	Treatment-3 (100% TAIWW)
Shoot length	cm	1.0(± 0.0001)	2.17(± 0.2886)****	2.34(± 0.5773)****	2.66(± 0.2886)**
Root length		3.83(± 0.2888)	3.33(± 0.5773)***	2.66(± 0.0001)****	5.17(± 0.2886)****
Seedling length		4.83(± 0.3464)	5.50(± 0.8660)****	5.00(± 0.0001)****	7.833(± 0.5777)****
Shoot fresh weight	g	0.04(± 0.0005)	0.05(± 0.0052)***	0.026(± 0.0011)****	0.062(± 0.0119)****
Root fresh weight		0.03(± 0.0045)	0.06(± 0.0144)****	0.018(± 0.002)***	0.057(± 0.0123)****
Seedling fresh weight		0.07(± 0.0045)	0.11(± 0.0077)****	0.044(± 0.0026)****	0.011(± 0.0065)****
Shoot dry weight		0.006(± 0.0005)	0.005(± 0.0005)**	0.006(± 0.0051)***	0.007(± 0.0051)****
Root dry weight		0.01(± 0.0047)	0.004(± 0.0017)*	0.008(± 0.0005)****	0.018(± 0.0002)****
Seedling dry weight		0.03(± 0.0048)	0.008(± 0.0036)****	0.015(± 0.0015)****	0.028(± 0.0005)***

The value outside the parentheses represents the mean, while the values in parentheses represent the standard deviation and standard error. The significance levels are indicated as follows: $*p < 0.1$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

DW, distilled water; UTAIWW, untreated agro-industrial wastewater; TAIWW, treated agro-industrial wastewater.

3.2.4. Morphological parameters observed for *L. esculentum*

In *L. esculentum*, seedling lengths (both shoot and root) were longer in TAIWW concentrations compared to UTAIWW concentrations. Similarly, the root and shoot weights of *C. annuum* were found to be higher in treated wastewater concentrations than in untreated wastewater concentrations (Table 5; Figures 9 and 10).

The average seedling length of *C. annuum* var. Astra was longer in TAIWW (shoot: 2.66 cm; root: 5.17 cm) than the seedling lengths (shoot: 2.17 cm; root: 3.33 cm) of untreated wastewater. Similarly, the average seedling length of *L. esculentum* var. Kristina was longer in TAIWW (shoot: 5.66 cm; root: 15.03 cm) than the seedling lengths (shoot: 4.33 cm; root: 10.60 cm) of untreated wastewater (Figure 6).

The average shoot and root fresh weights of *C. annuum* var. Astra were higher in treated wastewater (shoot: 0.062 g; root: 0.057 g) compared to untreated wastewater (shoot: 0.05 g; root: 0.06 g). The average shoot and root fresh weights of *L. esculentum* var. Kristina were higher in treated wastewater (shoot: 0.087 g; root: 0.02 g) compared to untreated wastewater (shoot: 0.062 g; root: 0.02 g) (Figure 7).

The average shoot and root dry weights of *C. annuum* var. Astra were higher in treated wastewater (shoot: 0.07 g; root: 0.018 g) compared to untreated wastewater (shoot: 0.005 g; root: 0.004 g). The average shoot and root fresh weights of *L. esculentum* var. Kristina were higher in treated wastewater (shoot: 0.018 g; root: 0.007 g) compared to untreated wastewater (shoot: 0.062 g; root: 0.02 g) (Figure 8).

Table 5 | Morphological parameters of *L. esculentum* under different treatments

Morphological parameters	Units	Control (DW)	Treatment-1 (UTAIWW)	Treatment-2 (50% TAIWW)	Treatment-3 (100% TAIWW)
Shoot length	cm	3.5(± 1.3222)	4.33(±0.577)****	3.16(±0.288)****	5.66(±0.5777)***
Root length		11.43(± 0.7505)	10.66(±1.527)**	9.33(±0.577)****	15.03(±0.057)****
Seedling length		14.93(± 1.0006)	15(±1.000)****	12.5(±0.500)****	20.7(±0.5196)****
Shoot fresh weight	g	0.034(± 0.0066)	0.062(±0.009)****	0.03(±0.003)**	0.087(±0.016)***
Root fresh weight		0.005(± 0.0047)	0.02(±0.003)***	0.006(±0.0017)****	0.021(±0.007)****
Seedling fresh weight		0.036(± 0.0141)	0.079(±0.0102)****	0.036(±0.0026)****	0.108(±0.0137)****
Shoot dry weight		0.018(± 0.0005)	0.011(±0.0005)****	0.011(±0.0020)***	0.018(±0.0005)****
Root dry weight		0.002(± 0.0009)	0.001(±0.0005)****	0.001(±0.0005)****	0.007(±0.0004)**
Seedling dry weight		0.020(± 0.0001)	0.013(±0.001)***	0.012(±0.0023)****	0.024(±0.001)*

The value outside the parentheses represents the mean, while the values in parentheses represent the standard deviation and standard error. The significance levels are indicated as follows: *$p < 0.1$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

DW, distilled water; UTAIWW, untreated agro-industrial wastewater; TAIWW, treated agro-industrial wastewater.

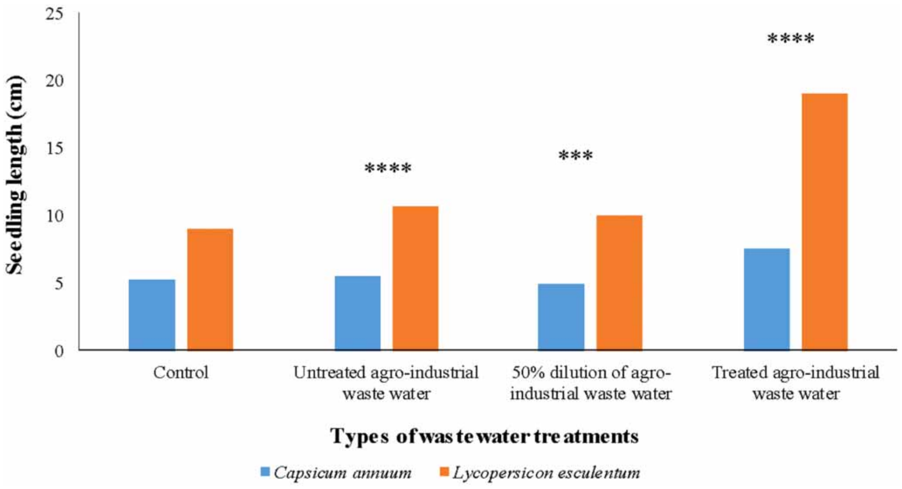


Figure 6 | Seedling length of *C. annuum* and *L. esculentum* under different wastewater treatments.

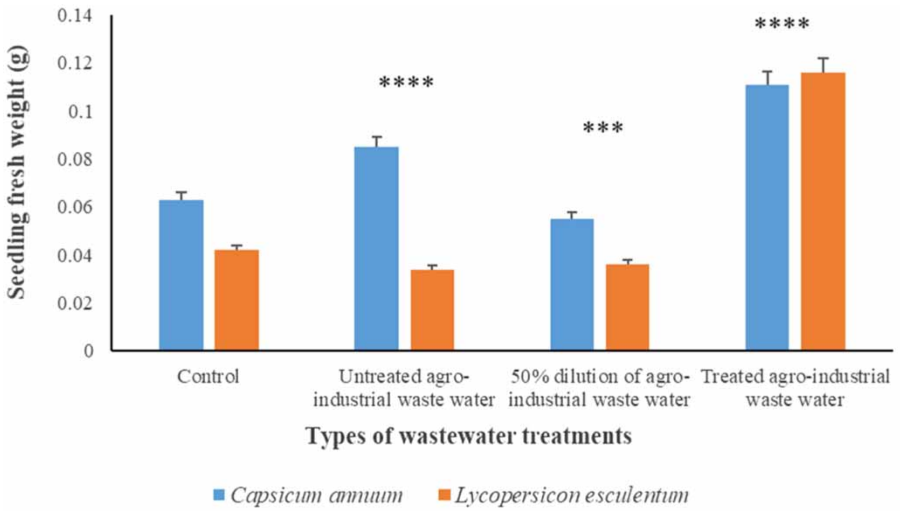


Figure 7 | Seedling fresh weight of *C. annuum* and *L. esculentum* under different wastewater treatments.

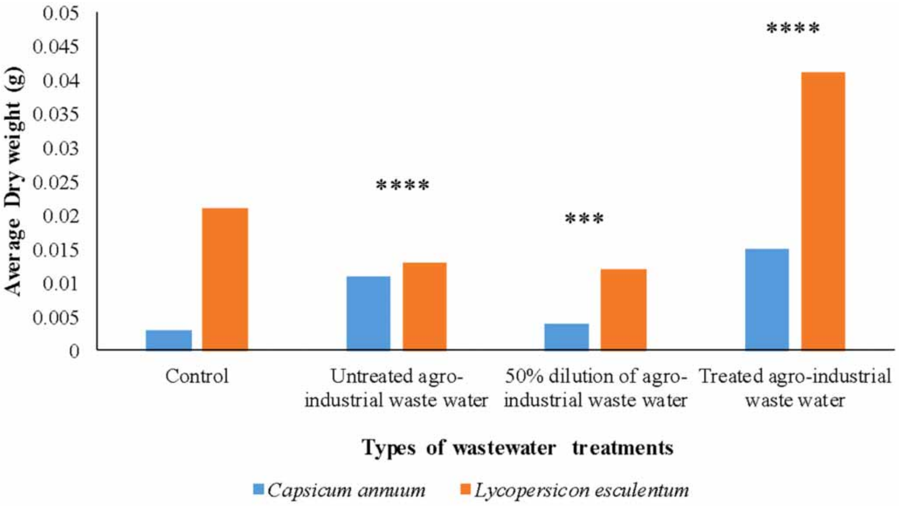


Figure 8 | Seedlings dry weight of *C. annuum* and *L. esculentum* under different wastewater treatments.

The seeds of *C. annuum* var. Astra and *L. esculentum* var. Kristina were germinated to assess the appropriateness of UTAIWW and TAIWW. The plates were incubated in a completely randomized design in a growth room under optimized conditions at 26 °C ± 1 (Figures 9 and 10).

Figure 9 | Germination of seeds of *C. annuum* after 21st days: (a) control, (b) raw untreated agro-industrial wastewater, (c) 50% dilution of untreated agro-industrial wastewater, and (d) treated agro-industrial wastewater.

Figure 10 | Germination of seeds of *L. esculentum* after 21st days: (a) control, (b) raw untreated agro-industrial wastewater, (c) 50% dilution of untreated agro-industrial wastewater, and (d) treated agro-industrial wastewater.

3.3. Plants nutritional analysis

An experimental trial was conducted to investigate and compare the effects of TAIWW and UTAIWW on the growth and production of *C. annuum* and *L. esculentum*. Furthermore, both vegetables grown under different water treatments were analysed for their nutritional components. A similar pattern of nutritional components was observed across the four different water treatments (Tables 6 and 7).

Table 6 | Nutritional components of *C. annuum* under different treatments

Nutritional components	Units	Control (DW)	Treatment-1 (UTAIWW)	Treatment-2 (50% TAIWW)	Treatment-3 (100% TAIWW)
Carbohydrates	%	3.21(± 0.021)	4.21(± 0.001)**	4.01(± 0.0045)***	4.20(± 0.0024)****
Proteins		2.12(± 0.0001)	3.50(± 0.002)****	3.12(± 0.0012)***	2.80(± 0.0013)****
Fats		0.26(± 0.00001)	2.61(± 0.003)****	3.20(± 0.00031)****	3.70(± 0.0067)**
Fibres		1.01(± 0.0001)	2.50(± 0.0005)***	1.06(± 0.0011)****	1.80(± 0.0091)****
Moisture		83.3(± 2.005)	84.55(± 0.0013)****	85.00(± 0.0201)***	85.00(± 2.005)****
Ash		2.24(± 0.001)	2.75(± 0.0023)****	3.61(± 0.051)****	2.50(± 0.0004)**
Energy	kcal/100 g	60.30(± 1.017)	54.33(± 0.002)***	57.32(± 1.006)*	61.30(± 2.001)****

The value outside the parentheses represents the mean, while the values in parentheses represent the standard deviation and standard error. The significance levels are indicated as follows: *$p < 0.1$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.
DW, distilled water; UTAIWW, untreated agro-industrial wastewater; TAIWW, treated agro-industrial wastewater.

Table 7 | Nutritional components of *L. esculentum* under different treatments

Nutritional components	Units	Control (DW)	Treatment-1 (UTAIWW)	Treatment-2 (50% TAIWW)	Treatment-3 (100% TAIWW)
Carbohydrates	%	5.23(± 0.0023)	5.01(± 0.005)***	4.99(± 0.0045)****	5.46(± 0.0067)**
Proteins		10.50(± 1.071)	10.67(± 0.455)****	9.79(± 0.0366)***	10.91(± 0.00234)****
Fats		0.02(± 0.0001)	0.26(± 0.0006)**	0.13(± 0.0008)****	0.12(± 0.0006)****
Fibres		0.01(± 0.0005)	0.13(± 0.0001)****	0.21(± 0.0005)****	0.11(± 0.0003)***
Moisture		80.87(± 6.231)	82.01(± 8.005)****	81.47(± 7.0456)****	82.18(± 8.018)****
Ash		3.37(± 0.0234)	1.92(± 0.0055)*	3.41(± 0.0234)**	1.22(± 0.0005)****
Energy	kcal/100 g	63.02(± 7.1231)	65.05(± 5.005)****	60.29(± 6.025)****	66.56(± 8.049)***

The value outside the parentheses represents the mean, while the values in parentheses represent the standard deviation and standard error. The significance levels are indicated as follows: *$p < 0.1$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.
DW, distilled water; UTAIWW, untreated agro-industrial wastewater; TAIWW, treated agro-industrial wastewater.

3.3.1. Nutritional analysis of *C. annuum*

The nutritional analysis of leaves of *C. annuum* showed a positive response to the applied treatments. Carbohydrates, proteins, moisture, fats, fibres, and energy content in the leaves recorded the highest significant values under biotreated agro-industrial wastewater (Table 6).

3.3.2. Nutritional analysis of *L. esculentum*

The nutritional analysis of the leaves of the *L. esculentum* showed a positive response to the applied treatments. Carbohydrates, proteins, moisture, fats, fibres, and energy content in the leaves recorded the highest significant values under biotreated agro-industrial wastewater (Table 7).

4. DISCUSSION

According to previously published studies (Sivaprakasam *et al.* 2008; Patel & Vashi 2010a, 2010b; Rashid *et al.* 2020), bioremediation using bacterial strains is a promising method for treating agro-industrial wastewater and preparing it for agricultural use. In the current study, *B. paramycoides* MT477810 was utilized for the biotreatment of agro-industrial wastewater, and the efficacy of fertigation using this biotreated water was investigated on the growth of *C. annuum* (var. Astra) and *L. esculentum* (var. Kristina). It was demonstrated that the biotreatment of wastewater samples significantly reduced the colour of agro-industrial wastewater, consistent with the findings of Hai *et al.* (2007). The biodegradability indices of our wastewater samples ranged from 0.3 to 0.6. According to the literature, a BOD/COD ratio greater than 0.3 indicates the need for biological treatment of wastewater (Wu *et al.* 2014), highlighting the necessity to treat the wastewater sample under study.

The activity of several bacteria is directly linked to decreases in the physicochemical parameters of wastewater, including pH, EC, salinity, turbidity, COD, BOD, TDS, TSS, and heavy metal concentrations following biotreatment. In this study, pH was decreased by 60.17%, EC improved by 22%, salinity by 16%, turbidity by 48%, COD by 40%, BOD by 73%, TDS by 62%, and TSS by 25%. Previous literature suggests that bacteria can metabolize basic or acidic substances, thereby neutralizing or reducing pH values. For example, certain species of *Bacillus* and *Pseudomonas* are well-known for modifying pH levels through their metabolic processes. The enzymes they produce can alter organic substances into less acidic or basic forms, which helps regulate wastewater pH (Mehrotra *et al.* 2021).

Similarly, bacteria can improve salinity and EC by breaking down salts and absorbing ions as part of their metabolic activities. For instance, sulphate-reducing bacteria can transform sulphate ions, which balances the total ionic concentration. Additionally, microbial treatments involving *Halomonas* species have been shown to mitigate high salinity levels by reducing salt content through ionic adjustments (Song *et al.* 2023). Bacterial bioremediation also facilitates turbidity reduction. Bacteria such as *Aeromonas hydrophila* and *Bacillus subtilis* achieve this by flocculating suspended particles, aiding their sedimentation, and improving water clarity. These bacteria produce bioflocculants, which help microbial cells adhere to floating materials, making removal more effective (Ho *et al.* 2022).

Bioremediation by bacterial species such as *Escherichia coli* and *Pseudomonas putida* also reduces BOD and COD by breaking down organic matter. These bacteria utilize organic compounds in wastewater as a carbon source, degrading complex organic molecules and lowering the oxygen demand needed to stabilize the water. Combining anaerobic and aerobic bacteria enhances the reduction of BOD and COD (Singh *et al.* 2024). Bacterial species also contribute to reducing TDS through the breakdown of dissolved organic and inorganic materials. For example, *Rhodococcus* and *Pseudomonas* species have demonstrated high efficacy in removing dissolved pollutants. These bacteria mineralize and metabolize various organic compounds, thereby lowering TDS levels (Peng *et al.* 2020).

For the removal of TSS, bacteria such as *Bacillus licheniformis* produce extracellular polymeric substances (EPS), which cause suspended particles to aggregate, simplifying sedimentation and also reducing turbidity (Gupta *et al.* 2023). Certain bacterial species, such as *Bacillus cereus* and *Pseudomonas aeruginosa*, are capable of biosorbing and accumulating heavy metals like lead, nickel, and chromium. These bacteria either store metal ions in their cell walls to prevent release into the water or use the metals for metabolic purposes. This biosorption process is particularly effective for heavy metal removal (Xie 2024). These results align well with previous studies (Rashid *et al.* 2020) and underscore the strong potential of bacterial strains for removing a wide variety of contaminants from complex wastewaters.

In comparison with untreated wastewater or diluted untreated wastewater, the present study showed that the germination parameters of both vegetable species improved significantly when irrigated with biotreated wastewater. The use of treated wastewater resulted in higher final germination percentages (FGPs), germination speeds (SGs), germination values (GVs), and germination indexes (GIs). The best germination outcomes were observed when *C. annuum* and *L. esculentum* were irrigated with treated wastewater. These findings are consistent with those of Aslam *et al.* (2023), who also reported enhanced germination with TAIWW.

Additionally, seedlings irrigated with biotreated wastewater exhibited larger shoots and roots compared to those irrigated with untreated wastewater. Specifically, under treated wastewater conditions, *C. annuum* seedlings developed shoot lengths of 2.66 cm and root lengths of 5.17 cm, while *L. esculentum* seedlings developed shoot lengths of 5.66 cm and root lengths of 15.03 cm. Similar results were reported by Singh *et al.* (2012), demonstrating that treated wastewater enhances root and shoot growth in various crops. These findings suggest that biotreatment with *B. paramycoides* not only removes harmful pollutants but also improves the nutrient profile of the water, promoting better seedling development.

The study also found that seedlings irrigated with biotreated wastewater had significantly higher fresh and dry biomass. The fresh biomass of *L. esculentum* and *C. annuum* was 0.108 and 0.011 g, respectively, while the dry biomass was 0.028 and 0.024 g. This aligns with the findings of Patel & Vashi (2010b), who reported increased fresh and dry biomass in crops irrigated with biotreated wastewater. In contrast, seedlings irrigated with untreated wastewater showed significantly lower biomass, highlighting the importance of biotreatment in promoting plant growth.

By analysing the growth parameters (seedling length, shoot, and root biomass), it can be concluded that the differences in these values (up to 50%) were due to the biotreatment with *B. paramycoides* MT477810. This is particularly noteworthy, as the same amount of treated and untreated wastewater, containing different concentrations of macro- and micronutrients, was applied to the plants. Thus, the biotreatment likely enhanced the nutrient profile of the wastewater. These results are consistent with those of Motuzas *et al.* (2017), who observed that bioremediation improves soil nutrient availability, fostering healthier crop development. Furthermore, this bacterium is capable of biodegrading organic pollutants and potentially mineralizing them into simpler forms, such as nitrates, phosphates, and other essential nutrients required for plant growth (Rashid *et al.* 2020, 2021).

The results of the current study are supported by Mainardis *et al.* (2022), who found that fertigation with treated wastewater increases crop productivity and soil fertility. Fertigation is a safer and more sustainable choice for long-term agricultural use, as biotreated wastewater not only increases seedling biomass and length but also reduces the levels of metals and other contaminants in the soil. Bioremediation decreases the concentration of sediments and pollutants in wastewater, making the treated water more suitable for fertigation and enabling its broader application. Moreover, the treatment process enhances the availability of nutrients, which can be directly absorbed by crops, leading to improved growth outcomes. This is particularly important in arid regions like Pakistan, where sustainable agriculture depends on the efficient use of available resources.

5. CONCLUSION

Effective wastewater management is crucial, as water pollution poses a serious threat to both aquatic and terrestrial ecosystems. This study demonstrated that bioremediation of agro-industrial wastewater using bacterial isolates is an easy, cost-effective, and environmentally responsible alternative. Key physicochemical parameters, such as pH (60%), EC (22%), salinity (16%), turbidity (48%), COD (40%), BOD (73%), TDS (62%), and TSS (25%), were significantly reduced through bacterial treatment. Furthermore, heavy metals like lead, nickel, and chromium were undetected in the treated wastewater. The bacterial strain used in this study showed an impressive 87% decolorization potential when tested on agro-industrial wastewater.

The use of biotreated wastewater in fertigation reduced phytotoxicity in *L. esculentum* var. Kristina and *C. annuum* var. Astra by nearly 50% compared to untreated wastewater. Additionally, plants irrigated with biotreated effluent showed an increase in nutrient-rich components by 12–48%, along with higher fresh and dry weights, which increased by 9–30%. These findings highlight the effectiveness of this treatment in promoting plant growth and improving nutritional quality.

Finally, using bacterial isolates for bioremediation not only reduces the environmental hazards associated with agro-industrial effluent but also enhances agricultural productivity. This method requires less energy, time, and chemical processing compared to traditional treatment methods, making it more sustainable and cost-effective. However, it is recommended that the agro-industrial sector adopt biological treatment processes to produce nutrient-rich, safe effluents suitable for fertigation. Additionally, an in-depth analysis of the produce from these crops is necessary for human risk assessment to ensure food safety.

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AUTHOR CONTRIBUTIONS

A.A. and S.A.M. proposed and planned this research work. A.A., A.R., and T.A. conducted this research. A.R., S.A.M., T.A., and L.C.C. analysed the data. A.A. and A.R. prepared the manuscript. S.A.M. and L.C.C. reviewed the manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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