Metal Resistant Enterobacter cloacae ZA14 Enhanced Seedling Vigor and Metal Tolerance through Improved Growth, Physiology and Antioxidants in Tomato (Solanum lycopersicum) Irrigated with Textile Effluents

Zainab Naseem 1, Muhammad Naveed 1,*, Hafiz Naeem Asghar 1 and Mansoor Hameed 2

1 Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan
2 Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan
* Correspondence: muhammad.naveed@uaf.edu.pk

Abstract: The presence of toxic heavy metals and dyes in textile wastewater is a serious problem contaminating vegetables by irrigation. This contaminated food upon consumption undermines human health and is lethal for human life. The endophytic bacteria have the ability to degrade textile dyes and remediate heavy metals. The purpose of the present investigation was to evaluate useful concentration levels of textile wastewater (TWW) for irrigation in combination with the endophytic bacterium Enterobacter cloacae ZA14 to remediate heavy metals for improving growth of the tomato (Solanum lycopersicum) plant. The tomato seedlings showed inhibited germination (52%); suppressed root length (55%) and shoot length (53%); declined RWC (47%); lowest CSI (34%); reduced MSI (32%); increased accumulation of heavy metals Cr, Pb, and Cd in roots and shoots; with decreased metal tolerance index; and rise in production of total thiols (57%) at use of 100% TWW without bacterial application. On the contrary, the supplementation of endophytic bacterium ZA14 showed improved germination (100%), a decline of 3 and 5% in root and shoot length respectively, increased CSI (13%), decrease in MSI (6%), reduced bioaccumulation of Cr (root 30 and shoot 56%), Pb (root 58 and shoot 65%), and Cd (root 21 and shoot 58%), total thiols (76%), when irrigated with 25% TWW. Hence, it is concluded that the irrigation with 25% TWW, along with the application of Enterobacter cloacae ZA14, may improve the growth of tomato by mitigating the phytotoxicity of dyes and heavy metals from textile wastewater.

Keywords: textile effluents; endophytic bacteria; toxic heavy metals; tomato; metal chelation; metal tolerance

1. Introduction

The massive expansion in the world’s population and industrialization has resulted in an alarming increase in the demand for fresh water, with estimates indicating that agriculture uses 70% of the available fresh water, industry uses 19%, and households use 11% [1,2]. By discharging textile effluents into aquifers, the textile industry has damaged the quality of fresh water [3]. Four million and five hundred thousand tons of dyes and/or related degraded materials are released each year [4], and because of their persistent, mutagenic, and carcinogenic characteristics, they have emerged as a major environmental issue.

Textile dyes having strong azo bonds, e.g., amino group and aromatic rings, are hard to degrade. This property makes them highly persistent [5]. Textile effluents are associated with additional possible contaminants besides colors, such as heavy metals, oil, dust, and chlorinated solvents [6]. Many harmful heavy metals are found in textile wastewater, including iron (Fe), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), zinc (Zn), and copper (Cu) [7], and are considered as toxic. Even at low concentrations, heavy metals impair environmental quality and have fatal effects on human health [8]. A heavy metal,
such as Cr, is used to fix the color to the fabric. Leather, synthetic, and natural fibers are treated with Cd, Pb, and Cr, which are carcinogenic \[9,10\]. Moreover, heavy metals like Pb released from other sources such as vehicles also get mixed with wastewater and pollute the environment when released illegally \[11\].

The release of untreated textile effluents into water resources negatively impacts aquatic life. The presence of dyes in water interferes with the absorption and reflection of solar light \[12\]. Increased dye effluent concentrations reduce oxygen availability and limit sunlight, which has an adverse effect on the biological processes in aquatic ecosystems \[13\], and causes eutrophication \[14\]. Plant germination can be suppressed by using textile effluents. The germination percentage of seeds, height, and survival rate of seedlings are the primary parameters that indicate healthy growth of plants after exposure to textile effluent \[15\]. In biological systems, in response to contaminants, hydroxyl radicals are produced that are the most damaging free radicals and have the capacity to destroy all biological molecules. In order to overcome oxidative stress caused by contaminants, the plant stimulates its radical scavenging system by the support of antioxidants enzymes such as catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) to restore the normal functioning of plants \[16\].

Plants irrigated with water containing high concentrations of dyes showed reduced growth \[17\]. An increased accumulation of proline was reported in plants, which is an indicator of textile toxicity \[18\]. Cadmium pollution in various plants is associated with Fe insufficiency \[19\], inducing chlorosis in plants due to suppressed activities of chloroplasts and production of chlorophyll pigments \[20\]. Lead (Pb) contaminated food upon consumption causes a variety of health problems, including cardiovascular, reproductive, gastrointestinal, and renal diseases \[11\]. Therefore, the use of untreated effluents for irrigation ultimately increases concentrations of heavy metals in the soil, which has negative effects on human beings, animals, plants, and microbes \[16\].

Among available approaches for remediation of textile effluents, biological techniques are cheap and eco-stable \[21\]. The biological approach involves the use of bacteria, fungi, plants, algae, and biological enzymes \[22\]. Endophytic bacteria reside inside the plant tissues and promote plant growth, possess the capacity to solubilize phosphate, and contribute to the provision of nitrogen. They also have environmental applications \[23\]. Endophytic bacteria are reported for their ability to degrade textile pollutants \[24,25\]. Native bacteria existing in metal-contaminated regions have developed resistance naturally towards heavy metals \[26\]. Dye-degrading bacteria isolated from textile wastewater irrigated soil also remediated Ni, Cd, Co, Zn, and Cr heavy metals \[27\]. Endophytic bacteria caused degradation of textile pollutants, resulting in increased growth of plants irrigated by textile wastewater \[22\].

The bacterial biomasses are good bio-sorbent materials \[28\] and have the ability to hamper the stress of heavy metals by a number of processes like production of exopolysaccharides, metal-phosphates, siderophore production, and by increasing the acidification of the rhizosphere. Extracellular precipitation, alterations of highly toxic metal to less toxic metal, as well as biosorption, are some of the mechanisms of bacteria to flourish well even in a metal-contaminated environment \[29,30\] All of these processes contributed to the immobilization of heavy metals in soil and decreased their availability to plants, resulting in improved plant growth \[31\].

Plant growth promotion, metal tolerance, and immobilization of heavy metals by bacteria have lately been documented to promote plant growth and inhibit metal uptake inside plants \[32–34\]. Under metal toxicity, the growth promoting metal-resistant bacteria offer various defensive mechanisms in plants such as increased P-solubilization, production of siderophore, production of phytohormone such as indole-3-acetic acid (IAA), and 1-aminocyclopropane-1-carboxylate (ACC)-deaminase to promote growth with reduced metal uptake within plant tissue \[35–37\].

Tomato (Solanum lycopersicum) is an important vegetable crop in Pakistan and throughout the world due to its widespread cultivation \[38\]. It is an important source of nutrients
such as vitamins A and C, potassium, fibers [39], and high-functioning constituent compounds such as lycopene and ß-carotenes [40]. Tomato plants are highly sensitive to heavy metal toxicity [41]. Farmers in underdeveloped countries use wastewater containing textile effluents for irrigation due to the presence of nutrients such as Fe and Zn in the wastewater. Moreover, the reuse of wastewater ensures the availability of irrigation water all year round [42], and it is also beneficial for the management of wastewater. However, the presence of heavy metals and dyes makes the reuse of TWW unsuitable for irrigation of crops, and this problem needs to be addressed. A few reports are available on the use of different levels of TWW for plant growth with basic growth responses.

The prior inoculation of plants with endophytic bacterium to decrease the likelihood of textile wastewater toxicity; increase germination, improve growth, and limit metal uptake in plants is an ideal strategy that is not well-documented in the literature. Therefore, there is a need to study the comprehensive impact of using TWW with inoculation of beneficial endophytic bacterium to cope with the harms of textile effluents with sustainable use for an increase in plant growth. It is hypothesized that use of textile wastewater and the metal-tolerant endophytic bacterium, Enterobacter cloacae ZA14, may reduce the lethal impacts of TWW by decreasing the metal uptake for improved germination and growth to enhance the safe reusability of TWW for irrigation of crops. The objectives of the present study were to (i) evaluate suitable levels of application of textile wastewater for irrigation and (ii) explore the ability of endophytic bacterium to remediate toxicity imposed by textile effluents for improved seedling vigor and growth of tomato plants.

2. Materials and Methods

2.1. Bacterium Isolation and Identification

In this study, a total of twenty-seven bacterial strains were isolated from the plant Bauhinia variegata and wastewater (locally collected from city Faisalabad) samples and were grown on media supplemented with 200 mg L$^{-1}$ textile dye. The selected isolates were evaluated for dye decolorization assay and minimum inhibitory concentration of heavy metals (cadmium, lead, and chromium). The bacterial strain ZA14 showing prolific growth in both assays was further characterized for various plant growth-promoting traits. The selected bacterial strain was identified through amplification with polymerase chain reaction (PCR), sequencing, and bioinformatics analysis of its 16S rRNA gene sequence. For molecular identification, the DNA of selected isolate was extracted, and the 27F and 1492R primers were used for 16S rRNA gene sequencing [43]. The PCR reaction was carried out using 2.5 µL crude DNA as a template in total 25 µL reaction mixture according to the following program: 1 cycle of 4 min at 94 °C, 39 cycles of 1 min at 94 °C, 1 min at 55 °C, 1.5 min at 72 °C, and a final extension step at 72 °C for 5 min. The size of the amplified 16S rRNA was confirmed by separating on 1% agarose gel along with GeneRuler 1kb DNA (Fermentas, Germany). The 16S rRNA PCR product was purified using a PCR Purification Kit (Favorgen, Taiwan) and sequenced by Macrogen (Seoul, Korea). The 16S rRNA of ZA14 was compared with the known nucleotide sequences using BlastN accessed at http://www.ncbi.nlm.nih.gov/BLAST (last accessed on 18 September 2022). A phylogenetic tree was constructed by using software MEGA11 (Molecular Evolutionary Genetics Analysis version 11) [44], and the partial sequence was deposited in the GenBank database.

2.2. Preparation of Levels of Textile Effluents

The textile effluents were collected from different local textile units, mixed thoroughly, and stored at −4 °C. Characterization of TWW showed color (dark brown color), TSS (2748 mg L$^{-1}$), temperature (37 °C), odor (pungent), pH (8.9), EC (16.4), Cr (76.29), Pb (84.38 mg L$^{-1}$), and Cd (121 mg L$^{-1}$). The different treatments were prepared by mixing distilled water with TWW. 25% TWW was prepared by mixing 25 TWW and 75% distilled water. In 50% TWW, both textile wastewater and distilled water were mixed in equal volume. TWW (75%) was made by adding 75 TWW and 25% distilled water. In 100% TWW, the textile effluent was used in pure form.
2.3. Seed Inoculation

The inoculum of endophytic bacterium ZA14 was prepared in Luria-Bertani (LB) media in the Soil Environmental Microbiology laboratory, ISES, University of Agriculture, Faisalabad, by following the method of Naveed et al. [45]. The surface sterilized (0.1% HgCl₂ for 3 min and washed 5 times with distilled water) seeds were primed for 24 h with endophytic bacterium. The seeds without bacterial application were kept in LB media for 24 h.

2.4. Treatment Plan and Experimental Layout

Seeds of Solanum lycopersicum were obtained from Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The Sahel variety of tomato was used in the experiment. The different treatments (with and without ZA14 bacterium) used in the experiment were: T1 = Control, T2 = 25% TWW, T3 = 50% TWW, T4 = 75% TWW, and T5 = 100% TWW. Treatment T1 (with and without bacterium) treated with distilled water (DW) was kept as control.

A germination assay was conducted to evaluate the impact of different levels of textile wastewater on germination, early growth responses of tomato plants with the application of bacterium ZA14. Ten seeds of tomato plant (Sahel v.) were placed in petri-plates, lined with sterilized Whatman No. 1 filter paper, and each treatment was carried out in triplicate. The seeds were applied with different levels of textile wastewater. The germination related data was recorded from 1st to 8th day of sowing.

For the in vitro growth room experiment, three healthy seedlings from each treatment were transferred from petri-plate to plastic pot tray containing peat (bought from local market), and each treatment was carried out in triplicate. The seedlings were watered as per requirement and harvested three weeks after transplantation. The data of different growth and stress-related responses were observed.

2.5. Seedling Vigor Measurements

2.5.1. Germination Percentage (G %)

Germination percentage was observed by the method of Arnold et al. [46]:

\[
\text{Germination percentage (G\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100
\]  

2.5.2. Seed Vigor Index (SVI)

The seed vigor index was measured by method given by Abdul-Baki and Anderson [47]:

\[
\text{Seed vigor index (SVI \%)} = (\text{R.L + S.L}) \times G
\]

2.5.3. Germination Rate (G.R)

The Method of Maguire [48] was used to find germination rate, given as follows:

\[
\text{G.R} = \frac{\text{Number of seedlings}}{\text{Days to first count}} + \ldots + \frac{\text{No of seedlings}}{\text{days to final count}}
\]

2.5.4. Germination Index (G.I)

The germination index was measured by the formula [49] presented below:

\[
\text{G.I} = (7 \times n1) + (6 \times n2) + (5 \times n3) + (4 \times n4) + (3 \times n5) + (2 \times n6) + (1 \times n7)
\]

Here, n1 . . . n7 are the number of germinated seeds on first and subsequent days until the 7th day.
2.5.5. Promptness Index (P.I)

The promptness index [50] was calculated as followed:

\[
P.I. = nd2(1.00)+nd4(0.75)+nd6(0.5)+nd8(0.25)
\]  

(5)

Here, nd2, nd4, nd6, and nd8 are the number of seedlings’ emergence at 2nd, 4th, 6th, and 8th days.

2.5.6. Relative Seed Germination (RSG) and Relative Root Growth (RRG)

Relative seed germination and relative root growth were calculated by the previously reported method of Tiquia [51].

2.5.7. Root Toxicity (R.T %) and Shoot Toxicity (S.T %)

The root and shoot toxicity were estimated by the method of Idress et al. [50].

2.5.8. Percent (%) Inhibition of Germination

The percent (%) inhibition of germination was recorded by the formula [52], given as:

\[
\text{Percent} \% \text{ inhibition of germination} = 100 - \frac{\text{Germination index of treatment}}{\text{Germination index of control}} \times 100
\]

(6)

2.6. Measurement of Agronomic and Physiological Traits

Plant root and shoot length was measured by the help of a meter rod, and the data was recorded. The fresh and dry biomass of the plant was recorded on weighing balance and the data was recorded. The stem diameter was also measured. The relative water content (RWC) was measured by taking the fresh, turgid, and dry weight of leaf, by the following method [53]:

\[
\text{RWC}(\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100
\]

The 0.25 g fresh leaves were washed with distilled water and were homogenized with pestle and mortar with 5 mL acetone (80%). The samples were left overnight in refrigerator at 4 °C. After 24 h, samples were centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was taken, and the absorbance was measured with a spectrophotometer (Hitachi-U2001, Tokyo, Japan) at 663, 645, and 480 nm, respectively. Chlorophyll a (chl. a) and chlorophyll b (chl. b) were calculated following the method of Arnon [54] and chlorophyll stability index (CSI) was recorded by method of Sairam et al. [55].

2.7. Determination of Oxidative Stress Markers

The measurement of the membrane stability index (MSI) was carried out by the method of Almeselmani et al. [56] by using the formula:

\[
\text{MSI} = [1 - (C1 / C2)] \times 100
\]

Electrolyte leakage (EL) was measured according to Gong et al. [57] and calculated by the formula:

\[
\text{EL}(\%) = (EC1 / EC2) \times 100
\]

The superoxide anion content was recorded [58]. Crushing of 1 g plant material in 2% polyvinylpyrrolidone (PVP) and 0.5% Triton X-100 containing, 4 mL 50 mM phosphate buffer (pH—7.8) was done and spun at 10,000 rpm for 15 min. After removing the 0.5 mL supernatant in separate tube, 0.5 mL of phosphate buffer and 0.1 mL hydroxylamine hydrochloride was mixed. Incubations at 25 °C for 30 min were done. To this, 1 mL each of 1-naphthylamine and 3-aminobenzenesulphonic acid was supplemented and placed for 15 min at 26 °C. Optical density at 530 nm was recorded with spectrophotometer. The estimation of hydrogen peroxide content was performed by procedure given
by Velikova et al. [59]. A homogenized sample (100 mg) was centrifuged at the speed of 10,000 rpm for 20 min at temperature of 4 °C. The supernatant from extract was removed in the separate tube and mixed with 0.5 mL of potassium phosphate buffer and 0.5 mL KI. Absorbance was noted at 390 nm.

2.8. Determination of Antioxidant Activities

The catalase enzymatic activity was measured by following method of Chance and Maehly [60] with some changes. The sample solution for CAT estimation contained 3 mL of phosphate buffer, H$_2$O$_2$ and cell enzyme extract and absorbance were recorded at 240 nm. The activity of SOD was recorded by following the method of Giannopolitis and Ries [61]. The sample solution containing NBT (nitroblue-tetrazolium), riboflavin, methionine, phosphate buffer and cell enzyme extract was illuminated with 30W fluorescent lamps for 10 min. The absorbance was recorded at 560 nm. The contents of the polyphenol oxidase (PPO) enzyme were determined by the Kumar and Khan [62] method. The reaction ingredients were 50 mM potassium phosphate buffer with pH 6, 80 mM catechol, and plant sample extract. They were mixed and allowed to stand for 10 min, before H$_2$SO$_4$ (3.5 N) was added, and the absorbance was recorded at 495 nm.

2.9. Metal Chelating Compounds

To determine the total thiols, a tomato shoot sample was homogenized in a 20 mM ascorbate solution and centrifuged. The supernatant was separated, and to 0.5 mL of the resulting supernatant, 2.4 mL of 200 mM Tris HCl and 10 mM of DTNB were added. The mixture was allowed to settle for 20 min, and then, absorbance was recorded at 412 nm [63]. The nonprotein thiols of tomato plants were determined by the method of Del Longo [64]. The fresh shoots were macerated in ice-cold 5% sulfosalicylic acid, and then, centrifugation was done. To 100 µL of sample extract, PPB (potassium phosphate buffer, 0.1 M), 0.5 mL of 1 mM DTNB, and 0.5 M EDTA was added. It was then retained for 15 min, and the absorbance was recorded at 412 nm. Determination of the protein-bound thiols was done by subtraction of the nonprotein thiols (NPT) from total thiols (TT).

2.10. Metal Concentration in Plant Tissue

The plants were washed with water, oven-dried at 70 °C until a constant weight. Oven-dried samples were digested with the diacid method: reagents used were Nitric Acid (HNO$_3$) concentrated and Per-chloric Acid (HClO$_4$) concentrated at a 2:1 ratio. A mixture of acids (HNO$_3$ and HClO$_4$; 5 mL) was added in a ground plant sample and settled overnight. The digestion tubes were then retained on a hot plate for 30 min until the sample solution turned colorless. The cooled sample solution was filtered and, with distilled water, increased the volume of each sample up to 25 mL. The digested samples were fed to an Atomic Absorption spectrophotometer for the determination of heavy metals.

Translocation factor (TF) = M shoot/M root

Bioaccumulation = M (root + shoot)/ DW (root + shoot)

Single metal conc. = (Mroot × DWroot) + (Mshoot × DWshoot) / (Mroot + Mshoot)

where M is the metal concentration (mg kg$^{-1}$), and DW is the dry weight of the plant.

2.11. Data Analysis

Data were subjected to the normality of residuals using the Shapiro–Wilk test and analyzed using two-way ANOVA. Significant differences between the means were determined using the LSD test at a probability level of 5% [65]. Software by OriginLab corporation, version Origin Pro 2021 (64-bit: 9.8.0.200) (Northampton, MA, USA), was used for the preparation of figures.
3. Results

3.1. Identification of Bacterium

The selected strain was identified as *Enterobacter cloacae* ZA14 and provided with accession number OM570257 for nucleotide sequences submitted to GenBank. The phylogenetic tree was drawn from the gene sequences obtained from the identification of isolated bacterial strain ZA14 (Figure 1).

![Neighbor-joining tree based on 16S rDNA gene sequences showing relationships between Enterobacter cloacae ZA14 (accession No. OM570257) and related species Enterobacter cloacae subsp. cloacae 279-56 ATCC 13047 (accession No. NR 028912), Enterobacter cloacae subsp. cloacae strain DSM 30054 (accession No. NR 117679), Enterobacter cloacae NBRC 13535 (accession No. NR 113615), Enterobacter cloacae subsp. dissolvens LMG 2683 (accession No. NR 044978), Enterobacter cloacae subsp. dissolvens ATCC 23373 (accession No. NR 118011), Leclercia adecarboxylata CIP8292 (accession No. NR 104933), Enterobacter kobei 16SrRNA (accession No. LT547820), Enterobacter kobei 16SrRNA (accession No. LT547823), and Enterobacter kobei 16SrRNA (accession No. LT547821). The percentage numbers above each branch indicate the levels of bootstrap support for the branch point based on resamplings. The bar represents 0.002 substitutions per site.](image1.png)

**Figure 1.** Neighbor-joining tree based on 16S rDNA gene sequences showing relationships between *Enterobacter cloacae* ZA14 (accession No. OM570257) and related species *Enterobacter cloacae* subsp. *cloacae* 279-56 ATCC 13047 (accession No. NR 028912), *Enterobacter cloacae* subsp. *cloacae* strain DSM 30054 (accession No. NR 117679), *Enterobacter cloacae* NBRC 13535 (accession No. NR 113615), *Enterobacter cloacae* subsp. *dissolvens* LMG 2683 (accession No. NR 044978), *Enterobacter cloacae* subsp. *dissolvens* ATCC 23373 (accession No. NR 118011), *Leclercia adecarboxylata* CIP8292 (accession No. NR 104933), *Enterobacter kobei* 16SrRNA (accession No. LT547820), *Enterobacter kobei* 16SrRNA (accession No. LT547823), and *Enterobacter kobei* 16SrRNA (accession No. LT547821). The percentage numbers above each branch indicate the levels of bootstrap support for the branch point based on resamplings. The bar represents 0.002 substitutions per site.

3.2. Seedling Vigor

The application of bacterium in T2 showed 100% germination percentage (Figure 2A). Similarly, reduction in SVI of T2 showed the least impact of toxicity (5%) with ZA14 application, as compared to the control (ZA14) (Figure 2B). The most prominent reduction
in the germination rate (60%) (Figure 2C) and germination index (59.6%) (Figure 2D) was observed in T5 treatment without bacterial addition. The maximum value of relative seed germination (100) as presented in Figure 2E and relative root growth (96.7) (Figure 2F) with the lowest root toxicity (3.2%) (Figure 2G) and shoot toxicity (5.9%) (Figure 2H) was exhibited by T2 treatment when *Enterobacter cloacae* ZA14 was used. Maximum Tolerance index (97) (Figure 2I), and the lowest percent (%) inhibition of germination of −12.5 (Figure 2J) was displayed by T2 treatment when ZA14 bacterium was applied.

**Figure 2.** Impact of *Enterobacter cloacae* and textile effluents on germination, (A) germination percentage, (B) seed vigor index, (C) germination rate, (D) germination index, (E) relative seed germination, (F) relative root growth, (G) root toxicity, (H) shoot toxicity, (I) tolerance index, (J) promptness index, and (K) percent (%) inhibition of germination, of tomato seedlings. Treatments are presented as, T1 = DW, T2 = 25% TWW, T3 = 50% TWW, T4 = 75% TWW, and T5 = 100% TWW, without bacterium (WOB) and with bacterium (WB). Here ND represents not detectable. Data are presented as means of 3 replicates. Means sharing same letter do not vary significantly at $p < 0.05$.

### 3.3. Growth and Physiology of Tomato Plants

The least impact of TWW was observed in T2 with ZA14 application by showing decline of 5% in root length (Figure 3A), 3% in shoot length (Figure 3B), 9% in root fresh weight (Figure 3C), 4% in root dry weight (Figure 3D), 6% in shoot fresh weight (Figure 3E), 3% in shoot dry weight (Figure 3F), 5% in stem diameter (Figure 3G), and 2% in RWC (Figure 3H), as compared to T1 (with bacterium).

A maximum decline of 46% in chl a (Figure 4A) and 47% in chl b (Figure 4B) was found in T5 plants (without bacterium). In contrast to the control, the T2 plant showed a minimum decline of chl a (8%), chl b (14%), and total chl (10%) (Figure 4C) in the presence of *Enterobacter cloacae* ZA14, whereas T2 without ZA14 application showed a maximum increase in ratio Chl a/Chl b (9%) (Figure 4D). A 13% increase in CSI (Figure 4E) was indicated by T2 plants when endophytic bacterium ZA14 was applied.
Figure 3. Impact of Enterobacter cloacae and textile effluents on growth and physiology, (A) root length, (B) shoot length, (C) root fresh weight (D) root dry weight, (E) shoot fresh weight, (F) shoot dry weight, (G) stem diameter, and (H) RWC of 4-week-old tomato seedlings. Treatments are presented as T1 = DW, T2 = 25% TWW, T3 = 50% TWW, T4 = 75% TWW, and T5 = 100% TWW, without bacterium (WOB) and with bacterium (WB). Data are presented as means of 3 replicates. Means sharing same letter do not vary significantly at p < 0.05.

Figure 4. Impact of Enterobacter cloacae and textile effluents on chloroplastic pigments, (A) Chl. a, (B) Chl. b, (C) total Chl., (D) ratio Chl. a/Chl. b and (E) CSI of 4-week-old tomato seedlings. Treatments
are presented as T1 = DW, T2 = 25% TWW, T3 = 50% TWW, T4 = 75% TWW, and T5 = 100% TWW, without bacterium (WOB) and with bacterium (WB). Data are presented as means of 3 replicates. Means sharing same letter do not vary significantly at p < 0.05.

3.4. Oxidative Stress Markers in Tomato

The data exhibited 32% decreased MSI (Figure 5A) in T5 treatment without bacterium as compared to control, whereas the T2 treatment showed the least decline in MSI of 6% when Enterobacter cloacae ZA14 was applied. Among all treatments, T2 with ZA14 application showed minimum EL (−10%) as compared to the control (Figure 5B). The highest increase in superoxide anion (76%) (Figure 5C) and \( H_2O_2 \) (103%) (Figure 5D) was found in the T5 treatment without bacterial use.

![Figure 5. Impact of Enterobacter cloacae and textile effluents on oxidative stress markers, (A) MSI, (B) EL, (C) superoxide anion, and (D) \( H_2O_2 \) contents of 4-week-old tomato seedlings. Treatments are presented as, T1 = DW, T2 = 25% TWW, T3 = 50% TWW, T4 = 75% TWW, and T5 = 100% TWW, without bacterium (WOB) and with bacterium (WB). Data are presented as means of 3 replicates. Means sharing same letter do not vary significantly at p < 0.05.](image)

3.5. Antioxidant Activity in Tomato

The application of ZA14 bacterium has shown an improved assembly of antioxidants to alleviate ROS species generated in response to the TWW stress in tomato plants. The significant production of antioxidants was observed in treatment T5, as there was an increased production of SOD by 68% (Figure 6A); similarly, a 93% increased generation of CAT (Figure 6B) and 57% increase in PPO (Figure 6C) were recorded with the addition of Enterobacter cloacae ZA14.

3.6. Metal Chelating Agents

Textile effluent stress caused 33, 47, 62, and 76% rises in the total thiol contents in T2, T3, T4, and T5, respectively, with the application of ZA14 bacterium (Figure 7A). Similarly, the use of Enterobacter cloacae ZA14 in treatments T2, T3, T4, and T5 exhibited upgraded contents of non-protein-bound thiols by 37, 48, 65, and 83%, respectively (Figure 7B). The concentration of protein-bound thiols also increased in response to the increased toxicity of TWW with Enterobacter cloacae ZA14 in treatments T2, T3, T4, and T5 by 33, 47, 62, and 75%, respectively, (Figure 7C).
Figure 6. Impact of Enterobacter cloacae and textile effluents on antioxidant activities, (A) SOD, (B) CAT, and (C) PPO contents of 4-week-old tomato seedlings. Treatments are presented as T1 = DW, T2 = 25% TWW, T3 = 50% TWW, T4 = 75% TWW, and T5 = 100% TWW, without bacterium (WOB) and with bacterium (WB). Data are presented as means of 3 replicates. Means sharing same letter do not vary significantly at p < 0.05.

Figure 7. Impact of Enterobacter cloacae and textile effluents on antioxidant activities, (A) SOD, (B) CAT, and (C) PPO contents, of 4-week-old tomato seedlings. Treatments are presented as T1 = DW, T2 = 25% TWW, T3 = 50% TWW, T4 = 75% TWW, and T5 = 100% TWW, without bacterium (WOB) and with bacterium (WB). Data are presented as the means of 3 replicates. Means sharing the same letter do not vary significantly at p < 0.05.

3.7. Heavy Metal Accumulation and Tolerance Index

The least uptake of Cr was detected in T2 where 30 and 56% declined Cr uptake was found in the root (Figure 8A) and shoot (Figure 8B), respectively, with ZA14 bacterium. The treatment T2 showed 33% declined TF in tomato plants with the use of ZA14 bacterium (Figure 8C), 31% less bioaccumulation of Cr (Figure 8D), and 44% reduced the single Cr concentration (Figure 8E). The lead concentration in the roots (3.7 mg Kg\(^{-1}\)) and shoots (2.9 mg Kg\(^{-1}\)) reached a maximum in treatment T5 without bacterium (Figure 8F,G). The data revealed the lowest TF in T2 (0.351) with ZA14 bacterium (Figure 8H). The minimum bioaccumulation of 3.5 (Figure 8I) and 0.61 mg Kg\(^{-1}\) of single Pb concentration (Figure 8J) was recorded in T2 with the used ZA14 bacterium. The T5 showed a reduced uptake of Cd by 17% in root (Figure 8K), 38% in shoot (Figure 8L), 25% in TF (Figure 8M), 3% in bioaccumulation (Figure 8N), and 32% in single plant Cd concentration (Figure 8O), as compared to plants of T5 without endophytic bacterium. Among all treatments, the reduced concentration of Cd in root (21%), shoot (57%), TF (46%), the least accumulation of Cd (10.65) was found in the T2 treatment with bacterial use. Among all the treatments, the T2 plants with bacteria ZA14 showed the maximum MTI (0.96), as illustrated in Figure 8P.
Figure 8. Impact of Enterobacter cloacae and textile effluents on metal contents, (A) Cr in root, (B) Cr in shoot (C) Cr TF, (D) Cr bioaccumulation, (E) single Cr concentration, (F) Pb in root, (G) Pb in shoot (H) Pb TF, (I) Pb bioaccumulation, (J) single Pb concentration (K) Cd in root, (L) Cd in shoot (M) Cd TF, (N) Cd bioaccumulation, (O) single Cd concentration, and (P) MTI of 4-week-old tomato seedlings under textile phytotoxicity. Treatments are presented as, T1 = DW, T2 = 25% TWW, T3 = 50% TWW, T4 = 75% TWW, and T5 = 100% TWW, without bacterium (WOB) and with bacterium (WB). Here ND represents not detectable. Data are presented as means of 3 replications. Means sharing the same letter do not vary significantly at p < 0.05.

4. Discussion

The continuous supply of water and the presence of various nutrients like Fe, Ca, Mn, Zn, etc. in TWW, are the main reasons that force farmers to use wastewater for irrigation [66]. By providing organic matter content and micronutrients in small concentrations, TWW helps in the augmented growth of plants [67]. Previous reports also concluded that heavy metals are present in vegetables irrigated with TWW [68,69]. Metal accumulation in vegetables can be a menace to the health of human beings [70]. Consequently, the presence of dyes, high salt concentrations, and heavy metals are the drawbacks of TWW when used for irrigation of crops. The application of endophytic bacterium is reported to increase plant growth under abiotic stress such as heavy metals [31], dyes [41]. Therefore, the present study indicated the impacts of different levels of TWW along with Enterobacter cloacae ZA14, to reduce the detrimental effects of TWW for increased growth of Solanum lycopersicum plants.

The tomato seedlings showed improved germination and growth at a lower level as compared to high levels of TWW (T5). Panda et al. [71] similarly found that rice
plants with reduced TWW levels had improved seed germination and seedling growth. The treatments receiving TWW showed a gradual decline in germination response with increasing levels of textile toxicity. Kaushik et al. [72] suggested that the industrial effluent causes high osmotic pressure and, hence, causes reduced germination. According to Baruah et al. [49], the negative effects of heavy metals Cd, Pb, and Cu were indicated by decreased germination percentage, percent (%) inhibition of germination, seed vigor index, and germination percentage in tomato, pea, and wheat seedlings. In the presence of Enterobacter cloacae ZA14, the results showed a positive response on tomatoes by using 25% TWW. The application of Enterobacter cloacae ZA14 with 25% TWW (T2 treatment) increased the germination percentage, seed vigor index, germination rate, germination index, RSG, RRG, root toxicity, shoot toxicity, tolerance index, and promptness index of tomato seedlings. Bacteria have certain properties such as P-solubilizing, production of phytohormones such as IAA, gibberellic acid (GA), nitrogen fixation, ACC deaminase production, and biofilm [31,73], which help in reducing the detrimental impacts of textile effluents while increasing plant germination and early growth of tomato seedlings.

The prolonged exposure of TWW reduced the growth and physiology of tomato plants. As the TWW levels rose, a drop in the stem diameter, fresh and dry weight, and root and shoot length was observed. The relative water content was reduced as a result of the decreased growth. TWW at lower concentrations (T2) exhibited a less negative effect on tomato plant physiology and growth than TWW at higher concentrations (T5). The lack of nutrients or their unavailability, as well as the presence of significant amounts of dyes, heavy metals, and salts in TWW irrigated plants, may all contribute to stunted plant growth [74,75]. Plant development [76] and relative water content were limited by the lack of nutrients and salts such as cadmium. Application of the endophytic bacterium Enterobacter cloacae ZA14 improved the growth and physiology of plants under the toxicity of TWW. Endophytic bacteria have been involved in increasing the nutrient availability by mechanisms such as P-solubilization, Zn-solubilization, nitrogen fixation, degradation of textile dyes and heavy metals by degradation, chelation, and immobilization [38,75]. Trichoderma sp. supported an increase in germination, plant biomass, and other growth-related parameters under arsenic toxicity in chickpea [77].

The present study demonstrated that use of TWW at higher concentration levels inhibited the concentration of chloroplastic pigments such as chl a, chl b, total chlorophyll, and chl a/chl b. Similarly, the plants treated with TWW alone resulted in reduced CSI and, hence, reduced photosynthetic activity. The presence of dyes and heavy metal salts are the main factors in the reduced production of photosynthetic pigments and reduced CSI. It was reported that textile dyes, salts, and heavy metals have been responsible for reduced chlorophyll contents when present at higher concentrations [77,78]. The presence of Cd has already been documented to hinder photosynthetic activity by modulating the production level of chlorophyll pigments, along with photosynthesis [79]. Houri et al. [80] also reported that, upon exposure of Cd, Cr, Pb, and Al, photosynthetic activities of Urginea maritima declined. The reduced content of chlorophyll is mainly due to the conversion of chlorophyll a into pheophytin a, which is mediated by replacing magnesium (Mg) by H-atom [81,82]. Under stressful conditions, chlorophyll molecules in plants undergo many biochemical changes such as oxidative-reductive processes, phaeophytinization, which is the increased content of pheophytin [83] in the plant body [84]. The cell wall and thylakoid membrane of the chloroplast lose their structure, resulting in the loss of chloroplastic pigments. By degrading chlorophyll pigments, Pb, Cd, and Cr are thought to be responsible for the impairment of enzymes such as Rubisco, -aminolevulinic acid dehydratase, and Chlorophyll synthase, which are involved in the synthesis of chlorophyll [85]. CSI was found to be declining with increasing levels of TWW. Baruah et al. [49] confirmed that CSI decreased with increasing levels of heavy metals (Cd, Pb, and Cu) in tomato, pea, and wheat plants.

The application of endophytic bacterium resulted in improved photosynthetic pigments and high CSI in tomato plants. Bacteria have the tendency to bind iron for the production of siderophore complexes, so as to make iron available to plants instead of
metal in the chloroplast. Moreover, bacteria can bind directly with heavy metals, reducing the bioavailability of metal ions to plants and, hence, increasing the production of photosynthetic pigments [86]. Application of the bacteria Klebsiella pneumoniae in V. mungo plants improved the chlorophyll content under heavy metal stress [87]. Rizvi et al. [88] reported increased the levels of chlorophyll pigments due to Azotobacter chroococcum inoculation in maize plants exposed to copper and lead heavy metals.

In the present study, the assessment of oxidative stress markers, including EL, MSI, production of super oxide anion, and H$_2$O$_2$, was performed to evaluate the impact of different levels of TWW on tomato and the significance of using endophytic bacterium. The data showed a gradual rise in oxidative stress markers with increasing levels of TWW, which indicated the rising level of toxicity inside plant cells, increased production of ROS, and reduced stability of the cell membrane. The excess of heavy metals in plants triggers oxidation reduction processes, resulted in the generation of hydroxyl radicals from hydrogen peroxide (H$_2$O$_2$) [89]. Sabir et al. [90] documented that, due to increased levels of heavy metals, a change in the composition of a plant’s DNA occurs, leading towards the increased generation of ROS [91] and, hence, detrimental for the stability of biomolecules [92,93].

The metal chelating compounds such as total thiol, non-protein-bound thiol, and protein-bound thiols were assessed to evaluate the efficiency of metal remediation inside plants by using Enterobacter cloacae ZA14 under different levels of textile effluents containing heavy metals. The metal chelating compounds increased gradually with the increasing concentration of TWW in tomato plants. The most pronounced increase was observed in tomato plants under inoculation of the ZA14 bacterium. Inside the plant body, the mechanism of chelation is functional to detoxify detrimental substances such as heavy metals [94]. The removal of metals by chelation is basically done by the thiol content, followed by vacuolar compartmentation of metal-SH complexes [94,95]. Metal stress in plants ended with increased levels of thiols in various plants such as tobacco [96], barley [97], wheat [98], peas [99], maize [78], and tomato [41].

The increased content of metal chelators is regarded as the primary defense of plants [100]. The contents of thiols were found to be increased with an increasing concentration of metal [78]. Meanwhile, the supplementation of Enterobacter cloacae ZA14 also caused increasing levels of thiols in tomato plants with an increasing level of TWW. Our results are in accordance with the findings of Khanna et al. [41] that, with the inoculation of bacteria, metal-chelating compounds were increased in tomato under cadmium toxicity. In addition, non-protein-bound thiols are involved in the antioxidant production in plants. The most prominent thiol metabolites are cysteine and glutathione. Awasthi et al. [101] observed elevated levels of non-protein-bound thiols, glutathione, and phytochelatins in rice plants under arsenic toxicity by the inoculation of P. putida. This rise in thiol content might be due to the complexation of arsenic in roots and shoots of rice and due to improved uptake of nutrients such as nitrogen, zinc, phosphorous, and sulphur in the association of microbes that triggered the production of these metal chelators.

The present study revealed that the supplementation of Enterobacter cloacae ZA14 helped in the reduction of uptake of heavy metals (Cr, Pb, and Cd) with improved metal tolerance in Solanum lycopersicum plants when TWW was applied. Increased levels of Cr, Pb, and Cd heavy metals showed an increased uptake of metals in roots as compared to shoots. These results are also in line with the previous findings of various researchers. Rizvi et al. [88], who showed improved metal accumulation in roots of maize under copper stress, Awasthi et al. [101] showed higher levels of arsenic in roots of rice and rape seed under stress of Cd also displayed augmentation of the metal in roots [32]. The inoculation of bacterium in the present experiment lowered the heavy metal accumulation in roots as well as shoots, with lowered TF, bioaccumulation, and single metal concentration. Declined heavy metal uptake and translocation in aerial parts such as shoots is an adaptive strategy of plants growing in stress conditions [102].
The inoculation of endophytic bacterium Enterobacter cloacae ZA14 improved the metal tolerance in tomato plants even at higher levels of TWW. Madhaiyan et al. [103] revealed that tomato plants exposed to Ni and Cd stress had a lower metal tolerance index, but they also noted that Methylobacterium oryzae and Burkholderia sp. were effective with an increased metal tolerance index even at high metal concentrations. Heavy metals increased the production of the stress marker, ethylene, which declined due to the supplementation of these bacteria. Other mechanisms that bacteria utilize to remediate heavy metals include immobilization, precipitation of heavy metals by binding them with functional groups such as amides and hydroxyl groups, which lower their availability to plants. Bacteria are also responsible for chelation of heavy metals due to their ability to produce extracellular compounds such as exopolysaccharides, different metabolites, and protons that act as metal chelators and hence reduce the metal uptake in plants [88]. The use of the metal-tolerant strain B. megaterium reduced the translocation of the heavy metal Ni [104]. Arsenic-resistant Exiguobacterium by colonization of root surfaces caused decreased arsenic uptake in V. radiata plants [105]. According to a previous study [106], the production of indole-3-acetic acid and siderophores, as well as biofilm production, are adaptation strategies of bacteria to cope with heavy metal stress.

5. Conclusions
The application of TWW for irrigation of crops is a common practice by farmers in developing countries. The presence of textile dyes and associated heavy metals is the main cause of textile phytotoxicity. The inoculation of metal-resistant bacterium may reduce the harm of textile effluents, and this approach is environmentally sustainable. In the present study, we investigated the potential of metal-resistant endophytic bacterium to manage different levels of textile effluents for reduced phytotoxicity in tomato plants. The findings showed that increased levels of TWW caused increased textile phytotoxicity in tomato plants, whereas seed inoculation of tomato plants with Enterobacter cloacae ZA14 and exposure of 25% TWW exhibited improved germination and growth, reduced oxidative stress, and augmented the activity of antioxidants and noticeable metal chelation for reduced uptake of heavy metals in tomato plants. This indicates that lower levels of textile effluents can be managed effectively by Enterobacter cloacae ZA14 and are beneficial for irrigation practices. The farmer may use endophytic bacterium with textile wastewater to improve the growth of their crops. However, there is still a need to conduct studies on the field scale by using molecular approaches to further strengthen the knowledge of bacterial-mediated abiotic stress management in plants.

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