Water Air Soil Pollut (2026) 237:451
https://doi.org/10.1007/s11270-026-09124-w

Heavy Metals Pollution in Low Quality Water Irrigated Soil and their Impact on Bacterial Abundance and Diversity in the Rhizosphere of Swiss Chard (*Beta Vulgaris* L.) Seedlings

Bahati J. Manegabe · Titus AM. Msagati · Kikongo Marie-Médiatrice Ntabugi · Johannes P. Maree · Rian Pierneef · Karin De Bruyn · Maropeng V. Raletsena

Received: 21 July 2025 / Accepted: 9 January 2026 / Published online: 24 January 2026

Abstract Substandard irrigation water impacts the chemical characteristics of soil, which may subsequently modify the shape of soil bacterial communities. Five categories of water, including the river water (RW), acid mine drainage (AMD), untreated wastewater (UTWW), treated wastewater (TWW), and tap water (TW), were utilized as irrigation water samples. Soil and water samples were examined for heavy metals, such as Chromium (Cr), Cobalt (Co), Zinc (Zn), Arsenic (As), Cadmium (Cd), and Lead (Pb), using ICP-MS. The V1-9 region of bacterial 16S rRNA was PCR-amplified to evaluate the effects of heavy metals in low-quality irrigated soil on bacterial diversity and abundance in the rhizosphere of Swiss chard seedlings. Approximately 88.9% of heavy metals in water, with concentrations ranging from 0.03 to 432.8 mg/L, were detected at low levels in TW. Conversely, about 83.3% of heavy metals, with the concentrations between 0.38 and 553.78 mg/kg, were detected at low levels in TW irrigated soil (TS1). The electrical conductivity (EC), pH, and organic matter (OM) fluctuated based on the irrigation water and soil samples. Bacterial diversity and abundance in soils differed according on the quality of irrigation water samples. *Blastoccus*, *Microlunatus*, *Nocardioides*, *Solirubrobacter*, and *Streptomyces* exhibited higher relative abundance in soil subjected to low-quality water compared to soil irrigated with TW (TS1). Redundancy analysis (RDA) demonstrated that bacterial community structure in the rhizosphere of Swiss chard seedlings were influenced by heavy metals, EC, pH and OM. This indicates that the introduction of heavy metals into the soil can select sensitive bacteria, whereas soil OM can supply nutrients that enhances resistant/tolerant bacterial multiplication, thereby influencing seedling growth.

B. J. Manegabe (✉)
Department of Life and Consumer Sciences, University of South Africa, Pretoria, South Africa
e-mail: 47203420@mylife.unisa.ac.za

T. A. Msagati · J. P. Maree
Institute for Nanotechnology and Water Sustainability, College of Science Engineering and Technology, University of South Africa, Pretoria, South Africa

K. M.-M. Ntabugi
Department of Environmental Sciences, Université Officielle de Bukavu, South-Kivu Province, Bukavu City, Democratic Republic of Congo

R. Pierneef
Centre for Bioinformatics and Computational Biology, University of Pretoria, Pretoria, South Africa

K. De Bruyn
College of Agriculture and Environmental Sciences Laboratories, University of South Africa, Pretoria, South Africa

M. V. Raletsena
Department of Agriculture and Animal Health, College of Agriculture and Environmental Science Laboratory, Pretoria, South Africa

Keywords Bacterial community · Heavy metals · Irrigated soil · Irrigation water · Seedlings

1 Introduction

The reuse of low-quality water in agriculture is prevalent in arid and semi-arid countries in an attempt to mitigate the freshwater crisis and to increase food production (Krause et al., 2020; Obayomi et al., 2021; Shen et al., 2019). The scarcity of freshwater results from human activities and from reduced precipitation rates associated with the effect of climate change (Bastable et al., 2025; Manegabe et al., 2025a). With an increase in population growth to around 10 billion (Crovella et al., 2024) and the resulting food demand by 2050 is expected to reach 70% and 100% in developed and developing countries, respectively (Kousar et al., 2021). Within this context, the global demand of irrigation water will rise by 53%, including 50% in developing- and 16% in developed countries (Yerli et al., 2025). Consequently, reuse of low-quality irrigation water appears to be a viable option to mitigate fresh water crisis (Andleeb et al., 2023; Hashem & Qi, 2021). Currently, a land area of around 30 million hectares is watered with low-quality irrigation water (Heyde et al., 2025) and contributes to about 10% of the world's food supply (Khaskhoussy et al., 2022).

Agricultural reuse of low-quality water provides advantages within the broader framework of the circular economy for nutrient, organic matter, and water cycling (Heyde et al., 2025), promotes crop production throughout the year (Angelakıs et al., 2020; Wickramasinghe & Nakamura, 2025), and improves plant growth in low-fertile soil (Li et al., 2024; Manegabe et al., 2025a). The growth and production of plants in poor quality water irrigated soil may result from a substantial input of nutrients and organic matter, which potentially improves soil fertility (El-Khateeb et al., 2023; Espira et al., 2024; Soufi et al., 2025). Organic matter (OM) is crucial for soil health, stabilizes soil structure, and reduces the mobility and availability of toxic heavy metals (Yerli et al., 2025). In addition, OM may act as a potential buffer against pH fluctuations (Heyde et al., 2025), and may also provide an optimal environment for the survival of plants and their symbiotic bacteria (Moulia et al., 2023; Soufi et al., 2025). Despite those several benefits, low-quality irrigation water can contain pathogens (Crovella et al., 2024; Drane et al., 2024), heavy metals, antibiotics, and pesticides that may affect microbial community structure and plant health (García-Orenes et al., 2015; Cui et al., 2019; Mkhinini et al., 2020; Moulia et al., 2023).

The contamination by heavy metals in low-quality water-irrigated soil may pose significant ecological and phytotoxic risks due to their accumulation in the soil and their uptake by plants and symbiotic bacteria (Chen et al., 2021; Manegabe et al., 2025a; Ur Rahman et al., 2021). Heavy metals can affect plant physiological functions including photosynthesis, germinations, as well as water and nutrient balance in the plants (Manegabe et al., 2025a). In addition, heavy metal pollution in low-quality irrigation water may also alter soil bacterial community structures (Guedes et al., 2022; Guo et al., 2022). However, contradictions appear within these reported results. Thus, the reuse of poor-quality water in agriculture appears to lead to an increase in microbial diversity and abundance in irrigated soil (Cui et al., 2019; García-Orenes et al., 2015; Ibekwe et al., 2018; Krause et al., 2020). In contrast, Frenk et al. (2018) reported that the reuse of irrigation with poor-quality water did not significantly affect microbial community structures in irrigated soil. The mechanism of heavy metal toxicity results from their tendency to accumulate in microbial cells, leading to the generation of oxidative free radicals that damage cellular biochemical molecules, including DNA, proteins, and carbohydrates, particularly within cell membranes and organelles (Manegabe et al., 2025a; Mathivanan et al., 2021); to bind functional groups in the structure of cellular molecules (Pal et al., 2022); and to compete with essential cations for binding sites in enzymes for binding sites (Munir et al., 2022).

Soil bacteria provide vital ecological services in the rhizosphere of plants, including the decomposition of organic matter through biochemical cycles (Ding et al., 2023; Glick, 2014; Liu et al., 2019); protection of plants against pathogens (Glick, 2012); and the secretion of phytohormones, such as auxins, cytokines, gibberellic acid, 1-aminocyclopropane-1-carboxylate deaminase, abscisic acid, polyamines, and salicylic acid, which are crucial for plant growth and may also mitigate the adverse effects of ethylene on plants (Bae et al., 2016; Glick, 2014). In addition, soil bacteria may also produce the enzyme nitrate reductase NR1 and bZIP transcription factors (TGA1

and TGA4), which play important role in the nitrogen cycle (Ningombam et al., 2024). Many publications indicated that microbial secretions can reduce the mobility and bioavailability of heavy metals in irrigated soil, leading to the protection of plant and their symbiotic bacteria from the adverse effect of toxic metals (Alves et al., 2022; Manegabe et al., 2025a). Therefore, heavy metal contamination in irrigated soil may potentially alter microbial community structure, which in turn may severely impact plant growth and yield and then food security (Ameen et al., 2021; Ningombam et al., 2024).

Unlike organic pollutants, heavy metals are not easily degraded/destroyed in the soil (Li et al., 2020; Shen et al., 2019). The non-biodegradability of heavy metals is responsible for their long-term persistence and accumulation at high concentrations in the soil (Li et al., 2016; Moulia et al., 2023). Thus, heavy metals can exert a long-term selection pressure on soil bacteria by promoting gene transfer between exogenous water bacteria and resident soil bacteria to facilitate the adaptation of rhizosphere bacteria populations (Heuer & Smalla, 2007, 2012; Mkhinini et al., 2020). Furthermore, organic matter and nutrients released in low-quality water-irrigated soil may enhance the proliferation and development of soil bacteria (Cui et al., 2019; Guedes et al., 2022; Khaskhoussy et al., 2022) and may act as a buffer against metal toxicity and pH fluctuation to provide a suitable environment for bacteria survival (Heyde et al., 2025). Thus, the objective of this study was to evaluate the impact of heavy metal contamination in low-quality water-irrigated soil on the changes in bacterial diversity and abundance in the rhizosphere of Swiss chard seedlings.

2 Materials and Methods

2.1 Experimental Design

The experiment was performed in a greenhouse at the Science Campus of the University of South Africa (S 26° 10′ 30″ S, 27° 55′ 22.8″ E) using the soil collected from a farm in Johannesburg, South Africa. total of 450 tray cells (10 × 4 cm × 15 cm depth) were filled each with around 95 g of soil sample and later divided into five different groups of 30 cells. Then, the soil was subsequently moistened with tap water and gently compacted to 0.75 cm beneath the cell edges. Seeds acquired from the University of South Africa (UNISA) Horticulture Centre (HC) were disinfected for 10 min in a 10% H_2O_2 solution and subsequently rinsed several times with deionized water (Manegabe et al., 2025b; Si et al., 2021). The seeds were then sowed in tray cells, with one seed per cell at a depth of 0.5 cm below the soil surface, and then irrigated with tap water for ten days. Healthy seedlings from each replication (n = 20) were selected and treated with either river water (RW), acid mine drainage (AMD), untreated wastewater (UTWW), treated wastewater (TWW), or tap water (TW), which also served as the control.

2.2 Water Sampling, Physicochemical Properties and Heavy Metals Content

A total of five water samples of each type were collected in triplicate from each of the Braamfontein Spruit River (RW), Simbanye Gold Mining (AMD), Care untreated and treated wastewater plants (TWW and UTWW), and the University of South Africa (UNISA), Science Campus (TW). Irrigation water samples were collected in separate 5 L plastic containers. Prior to collection, bottles were initially decontaminated with 10% HNO_3, oven-dried at 60°C overnight, and repeatedly rinsed three times with the respective water sample before filling the container with the water sample (Manegabe et al., 2017). Water quality indices, such as pH and electrical conductivity (EC), were measured in situ using a Multimer Lovibond (SensoDirect 150) meter. Water samples designed for heavy metal analysis were digested according to U.S. EPA SW-846 (2007a) Method 3015A. Using this method, 15 mL of each replicate sample were introduced into separate microwave vessels containing an acid mixture of 5 mL of 70% HNO_3 and 1 mL of 35% HCl and maintained at 170°C for 20 min using a microwave system (Microwave Reaction System, Anton Paar Multiwave 5000).

2.3 Collection of Rhizosphere Soil, Heavy Metal and Physicochemical Analysis

Seedlings from the five treatment groups were randomly collected in replicate from the trays on days 26, 31, 36, 41, and 46 post-sowing. Those seedlings were used to collect triplicate rhizosphere soil

samples (n = 75) according to the method described by Xie et al. (2023). Using this method, composite soil samples from the control and the four experimental groups were stored in separate labeled glass bottles. The soil intended for DNA extraction was preserved at −20˚C until utilized. Soil samples, designed for physicochemical and heavy metal analysis, were air-dried at room temperature in the laboratory and then sieved with 2 mm mesh. The pH and EC were analyzed in a 1:2.5 (w/v) soil vs distilled water suspension (Batool et al., 2023). The organic matter (OM) was analyzed using the loss on ignition (LOI) method according to Li et al. (2020). Soil designed for heavy metals analysis was further oven at 75˚C for 72 h (Manegabe et al., 2025b) and digested according to the U.S. EPA (2007b) Method 3051A. In this procedure, 0.5 g of each composite soil sample was introduced into a microwave vessel containing an acid mixture of 10 mL of 70% HNO_3 and 3 mL of 35% HCl and digested at 175˚C for 15 min in a microwave system (Microwave Reaction System, Anton Paar Multiwave 5000).

2.4 Preparation of Standards and Heavy Metal Analysis

Prior for metal analysis, the digests of both water and soil samples were allowed to cool and centrifuged at 3,000 × g for 15 min. The supernatant was filtered through a 0.45 µm nylon filter syringe and diluted in ultrapure water. A multi-element stock solution of 100 µg/mL, supplied by the nanoparticle laboratory of the UNISA science campus in Florida, Johannesburg, South Africa, was used to prepare 200 mL of a 10 µg/mL stock solution that was diluted to 50, 75, 100, 125, 150, 200, 250, 500, 750, and 1000 ng/mL working solutions. The calibration standards, as well as water and soil samples, together with the blanks, were run in an Agilent HDC-ICP-MS (PerkinElmer Nexion 350D ICP-MS), equipped with an octopole collision cell and auto-sampler.

2.5 DNA Extraction

Microbial genomic DNA was extracted from each sample consisting of 0.2 g of rhizosphere soil using the ZymoBIOMICS DNA MiniPrep kit (Zymo Research) according to the manufacturer's protocol. The main steps in the extraction processes were as follows: (1) 0.2 g of a soil sample and 700µL of lysis buffer SL2 were added to MN bead tube type A to break open the cells. These were vortexed for 5 min at room temperature before being centrifuged at 11,000 × g for 2 min; (2) the supernatant from step 1 plus 150 µL of lysis buffer SL3 was added to a fresh Eppendorf tube and vortexed for 5 min to precipitate contaminant. Each tube was then incubated at 4˚C for 5 min, and centrifuged at 11,000 × g for 1 min; (3) the supernatant from each tube was then filtratered through a NucleoSpin® inhibitor Removal Column (red ring) by centrifugation at 11,000 × g for 1 min to remove PCR inhibitors; (4) each filtrate plus 250 µL binding buffer (SB) were added into a separate Eppendorf tube that was vortexed for 5 min and filtered through NucleoSpin® Soil Column (green ring) by centrifugation at 11,000 × g for 1 min; (5) each silicate membrane was washed by successively filtering 500 µL SB and 550 µL SW1 washing buffer through each NucleoSpin® Soil column tube by centrifugation at 11,000 × g for 30 s, before filtering twice with 650 µL SW2 washing buffer through each NucleoSpin® Soil column tube, followed by vortexing for 2 s, and then centrifuging at 11,000 × g for 30 s; (6) each silicate membrane was dried by centrifugation at 11,000 × g for 2 min before (7) load 30 µL of elution Buffer SE was loaded into each NucleoSpin® Soil column tube and centrifuging at 11,000 × g for 30 s to elute the membrane-bound DNA. The concentration of each extracted DNA sample was determined using a micro-UV spectrophotometer (Nano-drop One, Thermo Fisher Scientific, Waltham, MA). The extracted DNA was then stored at −20 °C being sent to Inqaba Biotechnical Industries for sequencing.

2.6 PCR Amplification and SMRT Sequencing

The bacterial 16S rRNA V1-9 region was amplified using barcoded universal primer pair 27F (12 × 27F) (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (8 × 1492R) (5′-TACGGYTACCTTGTT ACGACTT-3′) (Buetas et al., 2024) in a GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems™). The reaction mixture consisted of 2.5 µL of 10 × *Taq* buffer, 1 µL of 50 mM $MgCl_2$, 2.5 µL of 2 mM dNTPs, 0.2 µL of Platinum ® *Taq* Polymerase (5 U/µL, Invitrogen™), 5 pmol of 27F primer, 10 pmol of 1492R primer, and 8 ng of genomic DNA. The reaction volume was made up to 25 µL

with the addition of ultra-pure water. The PCR was performed under the following reaction conditions: The initial denaturation step at 96˚C min for 4 min was followed by 30 cycles of denaturation at 94˚C for 20 s, primer annealing at 57˚C for 30 s, and extension at 75˚C for 1 min followed by a final extension step at 72˚C for 10 min. The negative PCR control contained ultrapure water instead of template DNA. Purified PCR products were used for library construction using the SMRT bell Template Prep Kit 1.0 kit (Pacific Biosciences) according to the manufacturer's instruction and protocol. Qualification of the SMRTbell library was performed using the Fragment Analyzer (Agilent) and quantified using the Qubit HS dsDNA Assay (Thermo Fisher). The amplicons were sequenced using P6-C4 chemistry on a PacBio RS II instrument (Pacific Biosciences, USA). The quality of sequences was assessed using RS_ReadsOfInsert.1 (Yang et al., 2020). High quality 16S rRNA was selected based on the following criteria: (1) the number of sequencing of the insert ≥ 5; (2) the minimum prediction accuracy (90%); (3) the lowest and highest sequence length should vary between 1,400 and 1,800 nt, respectively (Yang et al., 2020).

2.7 Bioinformatics Analysis

Raw data were processed using by the RS_ReadsOfinsert.1 in the SMRT Portal, Version 2.7 (PacBio) protocol (Cao et al., 2017). The samples were analysed for bacterial diversity using QIIME2 (ver. 8.0) software (Gregory Caporaso, Flagsta, AR, USA) (Cui et al., 2022). The main steps in the bioinformatics process included: (1) removal of chimeric sequences in the representative set of OTU matrix using ChimeraSlayer software (version 1.7.0-dev (Haas et al., 2011); (2) raw sequence filtration, trimming, replication sequences, chimera removal, and amplicon sequence variant inference was performed using Pear (v0.9.6) software (Han et al., 2024; Yang et al., 2020); (3) high quality reads were aligned using PyNAST software (Caporaso et al., 2010); (4) the Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline (v1.16.0) was used to generate an amplicon sequence variant (ASV) table (Callahan et al., 2015); (5) the filterAndTrim function of the DADA2 pipeline was used to remove primers; (6) SILVA (v138.1) was used for taxonomic assignment using the dada2-formatted training files for taxonomy and assignment (Gurevich et al., 2013); (7) Operational Taxonomic Units (OTUs) were calculated from valid sequences using the Vsearch (v2.7.1) software uparse algorithm with a sequence similarity threshold of 97% (Cui et al., 2022); (8) Phyloseq (v1.24.2) was used to merge sample metadata, taxonomic assignment; (9) based on representative OTU sequences, Fast Tree software was used to calculate α-diversity indices (Shannon (H'), Chao1, ACE, and Simpson indices) to evaluate bacterial diversity in the samples (Cao et al., 2017; Haas et al., 2011; Han et al., 2024).

2.8 Statistical Analysis

The results of physicochemical properties, heavy metal content, and microbial community structures were presented as the mean $\pm$ SD of replicate samples. Statistical analyses were performed using SPSS software (version 21.0). One-way analysis of variance (ANOVA) was used to evaluate heavy metal concentrations and physicochemical parameters between irrigation water, irrigated soils, bacterial community structures in the soils. Data normality and homogeneity of variance were evaluated using Kolmogorov–Smirnov test and Levene's test. Since almost parameter data analyzed did not meet the parametric assumptions, the Kruskal–Wallis test was used to determine whether the values of parameters differed significantly across irrigated soils as well as between irrigation water. The difference was significant at a p-value less than 0.05 ($p < 0.05$). Redundancy analysis (RDA) was performed using the vegan package in R (Version 4.0.3) to investigate the correlation between the abundances of taxa, physicochemical parameters, and heavy metal content in irrigated soils (Table 1).

3 Results

3.1 Physicochemical and Heavy Metals Content in Irrigation Water

The results of physicochemical properties and heavy metal content in irrigation water samples are presented in Table 2. The AMD had the lowest pH (3.95 ± 0.86) and the UTWW was most alkaline ($pH = 9.28 \pm 3.03$). The pH (7.1 ± 0.08) in TW was higher than in RW and AMD. However, it was lower

Table 1 Initial physicochemical properties and heavy metals content in the soil

Parameters	Values	Standard
pH	7.04 ± 1.61	-
EC	341 ± 0.51	-
OM	3.8 ± 0.16	-
Cr	1421.893 ± 15.12	100 mg/kg
Co	208.271 ± 2.51	100 mg/kg
Zn	1161.729 ± 0.28	200 mg/kg
As	73.909 ± 1.19	5 mg/kg
Cd	1.067 ± 3.61	1 mg/kg
Pb	79.374 ± 8.34	60 mg/kg

Zn: Zinc; Cr: Chromium; Co: Cobalt; As: Arsenic; Cd: Cadmium; Pb: Lead, EC: Electrical conductivity, and OM: organic matter

compared to that in UTWW and TWW (Table 2). With the exception of the AMD, the pH in the irrigation water samples were alkaline and were within the WHO/FAO permissible limit set for irrigation water (Abegunrin et al., 2016; Atta et al., 2023). The electrical conductivity(EC) was relatively high and only the EC obtained for TW (EC = 171.8 ± 3.77 µS/cm) was lower and below the FAO standard (Atta et al., 2023). The highest EC (1973 ± 462.05 µS/cm) was shown by the AMD, followed by UTWW (EC = 618.2 ± 208.88µS/cm), and TWW (EC = 512.2 ± 38.61 µS/cm). A comparison between the pH or EC of the irrigation water type showed a significant difference ($p < 0.05$) (Table 2).

The concentrations of heavy metals, As, Cd, Cr, Co, Pb, and Zn, were determined in almost all irrigation water samples, and those varied in relation to the water sources (Table 2). Thus, about 88.9% heavy metals between were detected at low concentrations in TW, while the mean concentrations of Cr (46 ± 29.28 mg/L) and Zn (35.91 ± 3.56 mg/L) were relatively higher in RW. Additionally, the concentrations of Co (28.7 ± 6.77 mg/L) and As (269.19 ± 223.28 mg/L) were raised in AMD. The concentrations of Pb were relatively high in UTWW (4.3 ± 2.27 mg/L) and RW (4.19 ± 1.53 mg/L) (Table 2). With the exception of Zn and As, the concentrations of heavy metals did not exhibit significant differences ($p > 0.05$) between irrigation water. Furthermore, the concentration of Pb in almost irrigation water was below the FAO standards, whereas that of the remaining metals exceeded this limit (Abegunrin et al., 2016).

3.2 Physicochemical and Heavy Metals Content in Irrigated Soil

Table 3 presents the physicochemical properties and heavy metal content in the rhizosphere soil of irrigated Swiss chard seedlings. The pH (6.17 ± 0.12) was significantly lower in the soil irrigated with AMD (TS3), whereas the highest soil pH (7.2 ± 0.1) was noted in the soil exposed to UTWW (TS4). With the exception of TS3, the pH (7.06 ± 0.28) of the soil exposed to TW (TS1) was relatively lower than that in the other irrigated soil. The EC (1445.7 ± 100.0 µS/cm) was higher in the soil samples exposed to the AMD (TS3), whereas that in the soil irrigated with TW (TS1) was lower (EC = 273.33 ± 100.01 µS/cm). Furthermore, the level of OM (3.95 ± 0.12%) was found to be higher in TS4, whereas the lowest level of

Table 2 Physicochemical characteristics and heavy metal concentrations in irrigation waters

Water	pH	EC	Cr	Co	Zn	As	Cd	Pb
TW	7.1 ± 0.08b	171.8 ± 3.77d	3.53 ± 12.1ab	0.03 ± 0.6b	16.49 ± 2.74 c	0.06 ± 0.13b	0.07 ± 0.01a	1.33 ± 0.75a
RW	6.85 ± 0.74b	309.8 ± 14.65 cd	46 ± 29.28a	1.11 ± 0.8b	35.91 ± 3.56a	1.01 ± 0.74b	0.97 ± 0.14a	4.19 ± 1.53a
AMD	3.95 ± 0.86c	1973 ± 462.05a	12.37 ± 6.77ab	28.7 ± 6.77a	34.52 ± 2.91ab	269.19 ± 223.28a	1.17 ± 0.06a	2.81 ± 1.62a
UTWW	9.28 ± 3.03a	618.2 ± 208.88b	5.24 ± 6.14b	0.26 ± 0.26b	17.24 ± 9.22c	0.53 ± 0.62b	1.12 ± 0.16a	4.3 ± 2.27a
TWW	7.36 ± 0.35b	512.2 ± 38.61bc	30.47 ± 30.17ab	0.82 ± 0.74b	22.26 ± 12.01bc	0.57 ± 0.52b	0.92 ± 0.09a	3.52 ± 0.96a
p-value	< 0.001	< 0.001	0.179	< 0.001	0.017	0.027	0.087	0.740
FAO	6.5—8.4	250	0.1	0.05	2	0.1	0.01	5.0
South Africa	5.0–9.7	250	0.1–1	-	1–5	-	0.01–0.05	-

TW: Tap water; RW: River water; AMD: Acid mine drainage water; UTWW: Untreated water; TWW: Treated water; Zn: Zinc; Cu: Copper; Cr: Chromium; Co: Cobalt; As: Arsenic; Cd: Cadmium; Pb: Lead, EC: Electrical conductivity. Alphabetic letters denote significant results at p-value less than 0.05 ($p < 0.05$)

Table 3 Heavy metal content of seedling irrigated rhizosphere soil

soils		Physicochemical and heavy metal content in rhizosphere of Swiss chard seedlings							
	pH	EC	OM	Cr	Co	Zn	As	Cd	Pb
TS1	7.06 ± 0.28a	273.33 ± 1.01d	3 ± 0.16a	311.39 ± 15.32a	45.39 ± 1.19a	247.2 ± 14.17a	18.46 ± 0.42a	0.42 ± 0.02a	21.96 ± 0.19a
TS2	7.14 ± 0.08b	746.7 ± 111.54b	3.05 ± 0.16a	517.03 ± 38.12a	60.98 ± 5.03a	362.62 ± 35.34a	19.15 ± 3.61a	0.55 ± 0.06a	31.06 ± 2.61a
TS3	6.17 ± 0.12c	1445.7 ± 100.0a	2.09 ± 0.14a	453.5 ± 170.36a	72.76 ± 22.22a	353.1 ± 129.25a	38.17 ± 10.65a	0.38 ± 0.14a	29.02 ± 8.34a
TS4	7.2 ± 0.1ab	613 ± 113.66c	3.95 ± 0.12a	553.78 ± 10.09a	72.05 ± 2.51a	412.09 ± 21.54a	33.83 ± 1.49a	0.52 ± 0.06a	34.26 ± 1.41a
TS5	7.11 ± 0.06ab	557 ± 21.79b	3.25 ± 0.04a	521.6 ± 29.31a	66.96 ± 3.6a	328.35 ± 12.69a	31.9 ± 5.73a	0.46 ± 0a	32.49 ± 1.27a
p-value	0.002	< 0.001	0.138	0.081	0.128	0.106	0.792	0.071	0.092
FAO				100	100	200	5	1	60
Australia				50*		300*		3*	200*
Tanzania				100*		150*		1*	200*

TS1: soil irrigated with tap water, TS2: soil irrigated with river water, TS3: soil irrigated with acid mine drainage, TS4: soil irrigated with untreated wastewater, and TS5: soil irrigated with treated wastewater; Zn: Zinc; Cr: Chromium; Co: Cobalt; As: Arsenic; Cd: Cadmium; Pb: Lead, EC: Electrical conductivity, and OM: organic matter Alphabetic letters denote significant difference at p-value less than 0.05 ($p < 0.05$). * (Barakat et al., 2020)

OM (2.09 ± 0.14%) was noted in TS3. With the exception of TS3, the level of OM (3 ± 0.16%) was lower in TS1 compared to the other irrigated soils. Nonetheless, a comparison for OM did not exhibit significant difference ($p > 0.05$) between irrigated soils.

For heavy metals, the highest levels of Cr (553.78 ± 10.09 mg/kg), Zn (412.09 ± 21.54 mg/kg), and Pb (34.26 ± 1.41 mg/kg) were detected in TS4, whereas Cd (0.55 ± 0.06 mg/kg) was identified at high concentrations in TS2. The soil exposed to AMD exhibited the highest concentrations of Co (72.76 ± 22.22 mg/kg) and As (38.17 ± 10.65 mg/kg), whereas around 83.3% heavy metals were detected at low concentrations in TS1. Overall, the concentrations of Cr, Cd, and Pb in all irrigated soils were below FAO/WHO permissible limits set for irrigation water, whereas those of the other heavy metals were above it (Manegabe et al., 2025b) (Table 3). However, the comparison of heavy metal concentrations between irrigated soil did not show a significant difference ($p > 0.05$).

3.3 Soil Bacterial Community Diversity and Richness

Figure 1 illustrates biodiversity results of bacteria in the rhizosphere of Swiss chard seedlings exposed to water of different types. The present study found that the Chao1 index (748.565 ± 32.1) was relatively greater in TS4 than in the other irrigated soils. With the exception of TS4, the Chao1 (727.296 ± 35.2) in TS1 was higher than in the other irrigated soil. Additionally, the ACE (701.693 ± 139) index was higher in TS2 than in the other irrigated soils, while the lowest Shannon (H') index (0.918 ± 0.034) was relatively low in TS3. Furthermore, the highest Simpson index (0.987 ± 0.001) was relatively high in TS3 compared to the other irrigated soils.

3.4 Composition of the Bacterial Community in Soil Irrigated with Different Water Samples

The relative abundances of bacterial genera and phyla in the soil collected from the rhizosphere of Swiss chard seedlings exposed to different types of water were analyzed using 16S rRNA gene sequencing. The present study found that the most abundant genera were Amaricoccus (8.357 ± 1.713 −10. 064 ± 2.483%), *Bacillus* (12.22 ± 1.267–20.891 ± 1.857%), *Blastoccus* (10.591 ± 0.277–15.654 ± 1.198%), *Gemmatimonas* (8.77 ± 1.37–11.325 ± 0.916%), *Lyzobacter* (4.17 ± 0.54–6.884 ± 1.734%), *Microlunatus* (3.145 ± 1.146–5.071 ± 0.648%), *Microvirgas* (3.79 ± 0.89–4.928 ± 0.315%), *Nocardioides* (3.864 ± 0.654–5.24 ± 1.18%), *Pir4 lineage* (4.961 ± 0.578–6.613 ± 1.255%), *Pseudonocardia* (3.475 ± 0.28–4.146 ± 1.841%), *RB41* (4.5 ± 0.11–5.409 ± 1.157%), *Solirubrobacter* (5.245 ± 0.156–8.58 ± 0%), *Sphingomonas* (4.3 7 ± 1.31–7.896 ± 0.013%), and *Streptomyces* (3. 631 ± 2.116–5.54 ± 1.25%). Those genera presented 98.46, 95.67, 97.72, 98.6, and 97.6% of the total bacterial abundance in the rhizosphere of the Swiss chard seedling exposed to TW, RW, AMD, UTWW, and TWW,

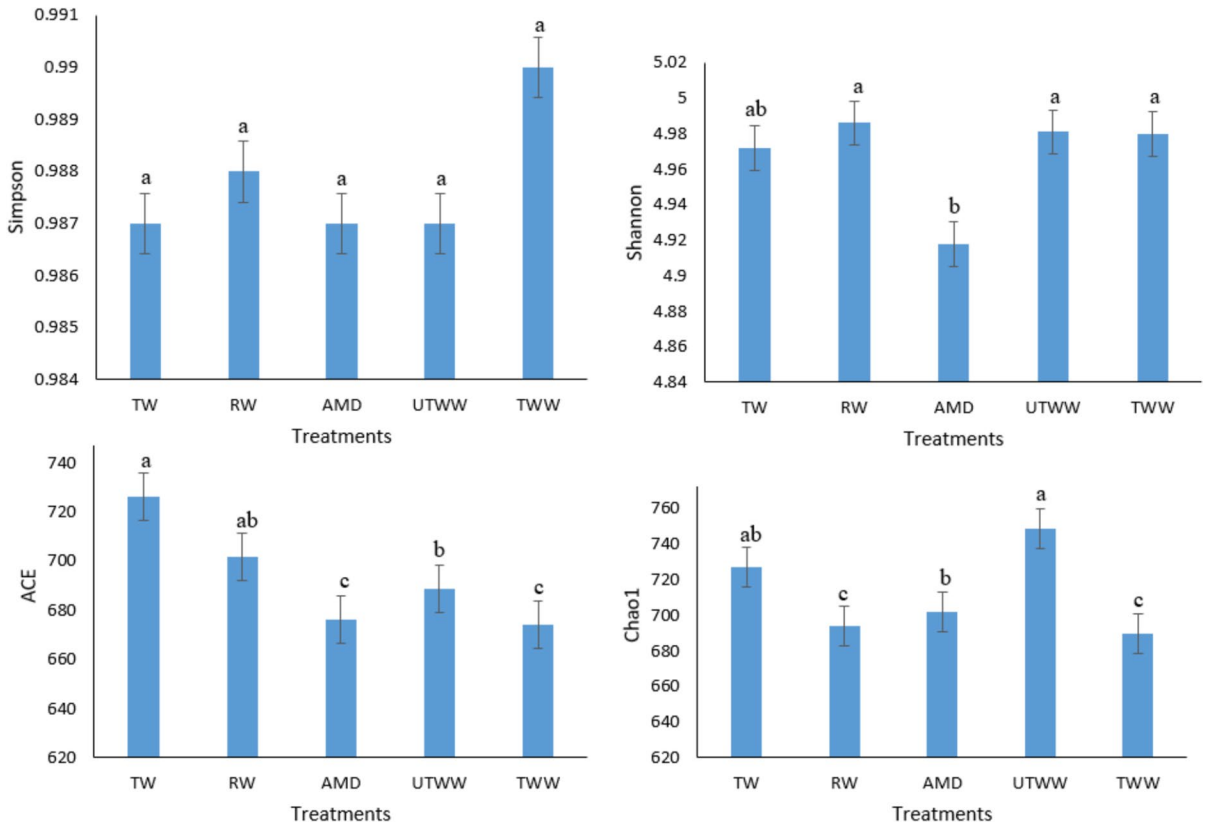

Fig. 1 Species richness Chao1 **A**, Shannon **B**, ACE **C**, and Simpson **D** in the rhizosphere of Swiss chard seedlings irrigated with tap water (TW), river water (RW), acid mine drainage (AMD), untreated wastewater (UTWW), and treated wastewater (TWW). Alphabetic letters indicate significant differences among irrigated soil at p-value less than 0.05 ($p < 0.05$)

respectively (Supplementary data, Table 1). Moreover, bacterial community composition of irrigated soil samples was different one another according to the quality of water used in irrigation. Indeed, *Maricoccus*, *Gemmatimonas*, *Microvirgas, and Sphingomonas* were abundant bacteria in TS1, whereas *Lyzobacter* and *RB41* dominated in TS2. Additionally, *Blastoccu* and *Microlunatus* were abundant in TS3; *Bacillus* and *Pir4 lineage* dominated in TS4, while *Nocardioides*, *Solirubrobacter*, and *Streptomyces* were abundant in TS5 (Fig. 2a). We noted that *Blastococcus, Microlunatus*, *Nocardioides*, *Solirubrobacter*, and *Streptomyces* were relatively abundant in low-quality water irrigated soils compared to TS1 (Supplementary data, Table 2). However, the abundance of bacterial genera did not reveal a significant difference ($P > 0.05$) between irrigated soils.

Furthermore, the present study indicated that bacterial phyla *Actinobacteriota* (21.489 ± 0.074–26.67 ± 0.136%), *Armatimonadota* (0.57 ± 0.015–0.728 ± 0.018%), *Bacteroidota* (3.92 ± 0.01–5.192 ± 0.007%), *Bdellovibrionota* (0.696 ± 0.007–1.068 ± 0.012%), *Chloroflexi* (6.344 ± 0.032–7.787 ± 0.003%), *Cyanobacteria* (0.346 ± 0.003–0.651 ± 0.029%), *Desufobacteriota* (0.126 ± 0.003–0.164 ± 0.002%), *Entothe onellaeta* (0.29 ± 0.005–0.442 ± 102%), *Firmicutes* (3.467 ± 0–5.65 ± 0.001%), *Gemmatimonadota* (4.181 ± 0.002–5.217 ± 0.004%), *Methylomirabilota* (0.329 ± 0–0.41 ± 0%), *Myxococcota* (2.882 ± 0.001–3.538 ± 0.005%), *Nitrospirota* (0.495 ± 0.101–0.654 ± 0.136%), *Patescibacteria* (0.77 ± 0.006 –1.121 ± 0.015%), *Planctomycetota* (6.851 ± 0.001–7.595 ± 0.016%), *Proteobacteria* (25.55 ± 0.101–28.764 ± 0.001%), RCP2-54 (0.369 ± 0.008

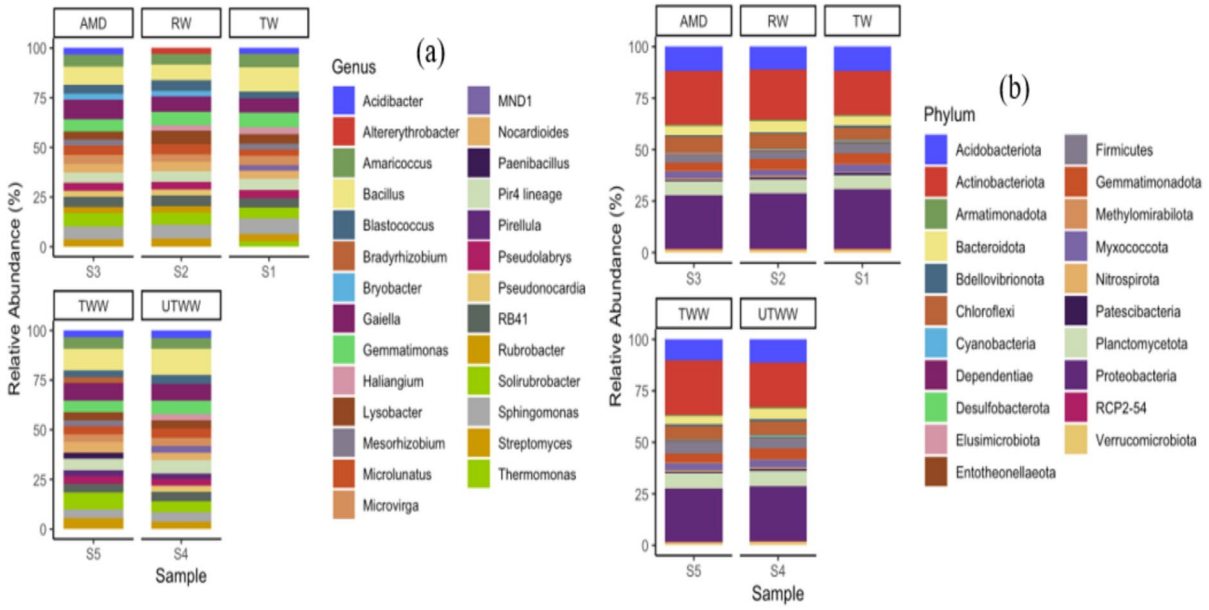

Fig. 2 Relative abundance of bacteria at genera level **a** and phyla level **b** in rhizosphere of Swiss chard seedlings exposed to tap water (TW), river water (RW), acid mine drainage (AMD), untreated wastewater (UTWW), and treated wastewater (TWW)

–0.424 ± 0.002%), *Verrucomicrobiota* (1.548 ± 0.012 –1.76 ± 0.003%) were the most dominant bacteria and these represented 88.22, 88.87, 88.2, 88.53, and 98.75% of the total bacteria in the rhizosphere of Swiss chard seedling irrigated with the TW, RW, AMD, UTWW, and TWW, respectively (Supplementary data, Table 2). In parallel to the genera, a relative difference was noted between bacterial phyla in the rhizosphere of Swiss chard seedlings exposed to water of different types. Consequently, around 44.4% of bacterial phylum between 3.475% and 20.891% was abundant in TS4. Additionally, *Armatimonadota*, *Gemmatimonadota*, *Myxococcota*, and *Proteobacteria* were dominant in TS1, while *Bacteroidota* and *Entotheonellaeta* dominated in TS2. Furthermore, *Chloroflexi* dominated in TS3, whereas *Chloroflexi*, *Firmicutes* and *Methyolomirabilota* were abundant bacteria in TS5 (Fig. 2b). However, the abundance bacterial phyla did not reveal significant differences ($p > 0.05$) between irrigated soils.

3.5 Redundancy Analysis

The impact of physicochemical parameters and heavy metals on soil bacterial composition at the genera (a) and phyla level (b) in the rhizosphere of Swiss chard seedlings exposed to irrigation water samples from different sources was evaluated using redundancy analysis (Fig. 3). The redundancy analysis (RDA) of the bacterial community at the genera level revealed that axes 1 and 2 explained 57.14% and 17.83%, respectively, of the total variation in the bacterial community (Fig. 3a). In axis 1, the major environmental abiotic variables that negatively influenced the soil bacterial community structure included As, Co, Cd, pH, and OM. In the axis 2, the bacterial community was affected negatively by OM, As, and EC, whereas the bacterial community was positively influenced by Pb, Co, Cd, and pH. At the phyla level, the RDA showed that axes 1 and 2 explained 50.89% and 38.49%, respectively, of the total variation in the bacterial community (Fig. 3b). In axis 1, the bacterial community in the rhizosphere of Swiss chard seedlings correlated positively with OM, As, Pb, Co, Cd, and pH. In axis 2, the bacterial community was positively influenced by pH, Cd, Co, Pb, and As, whereas on the negative side, it correlated negatively with OM and EC.

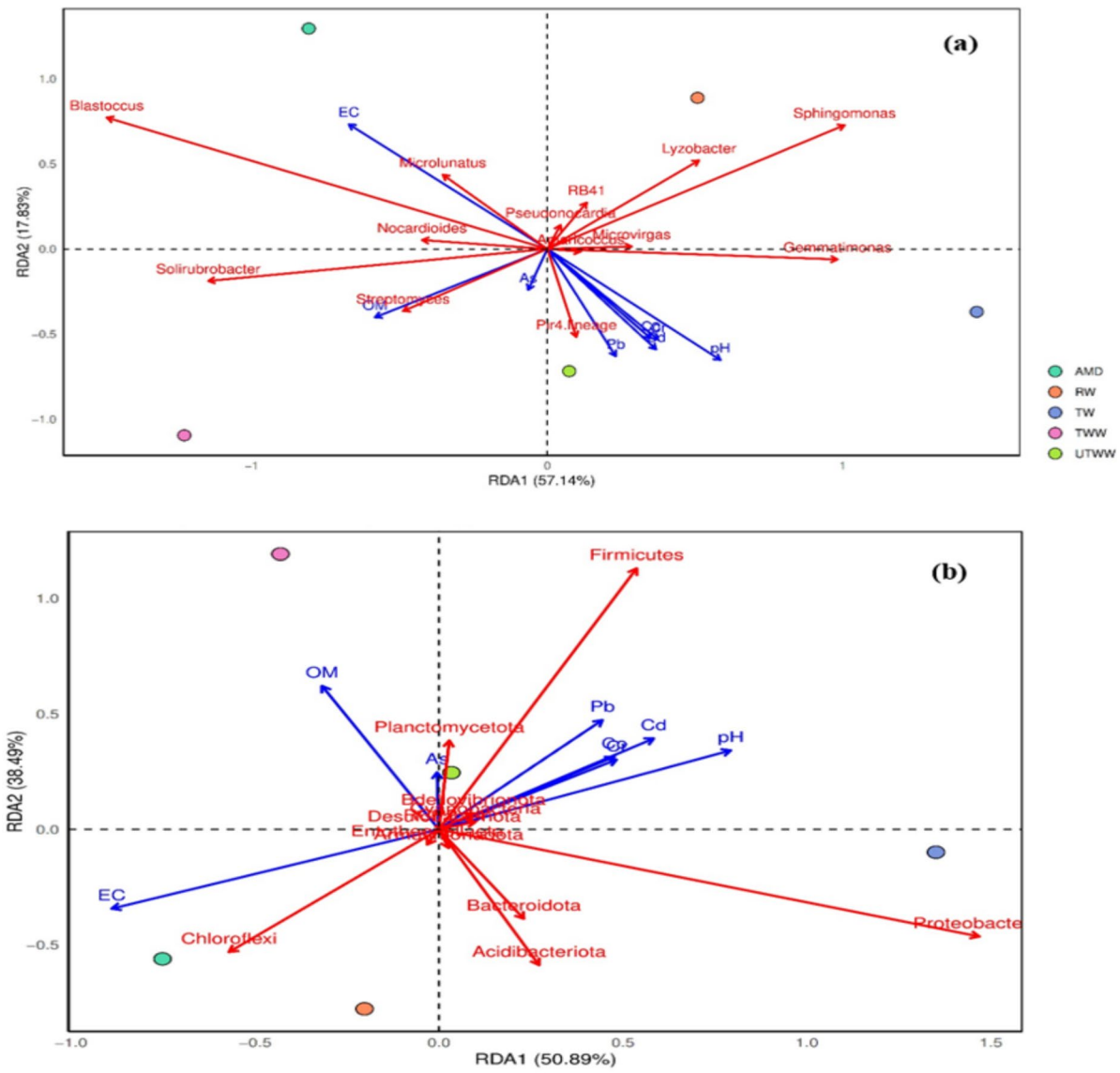

Fig. 3 Redundancy analysis shows the correlation between abiotic factors and bacterial community at genus **a** and phyla **b** level in the rhizosphere of Swiss chard seedlings. The dots represent irrigation water, including tap water (TW), river water (RW), acid mine drainage (AMD), untreated wastewater (UTWW), and treated wastewater (TWW)

4 Discussion

4.1 Heavy Metals Content and Physicochemical Properties of Water

The contamination of irrigation water by heavy metals poses a considerable global concern owing to their toxicity and accumulation in irrigated soil and plants (Andleeb et al., 2023; Angon et al., 2024). The present study found that about 88.9% of the heavy metals analyzed in irrigation water samples were detected at low concentrations in TW (Table 2). Throughout the samples, the concentrations of Cr and Zn were higher in RW, while those of Co and As were elevated in AMD, and Pb was detected at an elevated concentration in UTWW (Table 2). The concentrations of

heavy metals in water samples, with the exception of Pb, surpassed the FAO limit (Table 2). Consistent with our results, Hassan et al. (2024) have found that the concentrations of heavy metal in textile wastewater samples were elevated than in groundwater. A study by Ullah et al. (2022) reported that the concentrations of heavy metal in irrigation wastewater increased in the order of Fe > Cr > Pb > Co > Mn > Ni, while those in well water increased in the order was Fe > Cr > Co > Ni > Pb > Mn. Sharafi et al. (2022) evaluated heavy metals (Fe, Zn, Cu, Mn, Pb, Cd, Cr, Ni, and As) in irrigation water. They found the levels of heavy metals in river water were higher than in treated wastewater and well water. Heavy metal contamination in irrigation water has been reported in Iran (Sharafi et al., 2022), South Africa (Malan et al., 2015), and the Democratic Republic of Congo (Ngweme et al., 2020). A comparison of our results with previous findings indicated that the concentrations of Cr, Mn, Fe, Cu, and Zn in experimental water samples exceeded those reported in India (Guadie et al., 2021) Egypt (Khan et al., 2023), and Ethiopia (Singh et al., 2024). However, Pb concentrations in all water samples, including the control, were low compared to that detected in irrigation water in Ethiopia (Singh et al., 2024). Similarly, the concentration of Cd in irrigation was low that that reported in irrigation water in Pakistan (Khan et al., 2023).

For physicochemical properties, the pH in AMD was lower than the lower limit set by the WHO/FAO (Atta et al., 2023) and by South Africa (Ogugua et al., 2022) for irrigation water, whereas the pH of the other irrigation water sources fell within the acceptable range (Table 2). The decrease in the pH in irrigation water indicates the presence of free protons (Abbas et al., 2020). Similar to our results, Hatar et al. (2013) noted low pH in AMD in Malaysia gold mining. The decrease in water pH may result from the discharge of acid into irrigation water (Manegabe et al., 2025b). It is also reported that the decrease in water pH may result from the decomposition of OM through biochemical cycles (Abegunrin et al., 2016). In this study, the pH of UTWW was higher and exceeded the maximum FAO limit set for irrigation water, but it was within South African standard (Table 2). Consistent with our findings, Rahman et al. (2021) have also reported elevated pH in wastewater. The elevated pH in irrigation water may be attributed to a substantial quantity of OM in water (Abou-Tammame et al., 2022; Yerli et al., 2025). Alkaline pH was reported in irrigation wastewater in Pakistan (Abbas et al., 2020), Tunisia (Sdiri et al., 2023), India (Charan et al., 2023), and Bangladesh (Rahman et al., 2021).

The electrical conductivity (EC) of particular AMD was significantly higher and surpassed the FAO and South African limit set for irrigation water (Manegabe et al., 2025b; Ogugua et al., 2022), while that of TW was lower and below the standard (Table 2). Consistent with our findings, Verma et al. (2023) indicated that the EC in industrial and household irrigation wastewater was higher compared to tap water. Many publications have reported that the EC of water samples may fluctuate depending on the water sources (Abbas et al., 2020; Charan et al., 2023; Singh et al., 2024). The increased EC in irrigation water may be ascribed to dissolved salts and minerals, including Ca^{2+}, Mg^{2+}, Cl^{-}, Na^{+}, Mn^{2+}, PO_4^{3-}, and SO_4^{2-} in water (Agoro et al., 2018; Atta et al., 2023), but also to low OM level in water (Ribeiro de Sousa et al., 2014).

In parallel to AMD, that the pH in soil exposed to AMD (TS3) was lower, whereas that of the other irrigated soils, including the control, was alkaline, with the most alkaline being noted in the soil exposed to UTWW (TS4) (Table 3). These findings align with those of other researchers who have reported that soil pH may be affected by the quality of irrigation water (Manegabe et al., 2025b; Singh et al., 2024). The decrease in soil pH may result from OM decomposition through biochemical cycles in the soil (Yerli et al., 2025) and from the influx of protons (H^{+}) in the soil through irrigation water (Hatar et al., 2013). Furthermore, a report indicated that heavy metal pollution in the rhizosphere of plant can induce excessive secretion of root exudates comprising free protons (H^{+}) and organic acids, which can substantially decrease the pH of the soil (Alves et al., 2022). The low soil pH may increase the availability and mobility of heavy metals, which can adversely affect seedling physiology and bacterial eco-services in the rhizosphere (Yerli et al., 2025; Manegabe et al., 2025a). However, the increase in the soil pH may be attributed to OM influx in the soil through irrigation water (Yang et al., 2020) and from the discharge of basic cations, including Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} in the soil through low-quality irrigation water (Bouaroudj et al., 2019). Consequently, free protons (H^{+}) can bind free

electrons of OM and basic cations via electrostatic interactions, resulting in an elevation of soil pH and reduced mobility and availability of heavy metals (Khaskhoussy et al., 2022).

The EC of soil irrigated with the AMD (TS3) was higher than that of the other irrigated soil (Table 3). These results align with those published by Abegunrin et al. (2016) and Singh et al. (2024). They found that the EC of soil varied according to the quality of the irrigation water. The EC of the soil may be affected by the volume of water absorbed by the plant, the rate of evaporation, and the quantity of dissolved salts and minerals introduced in the soils by irrigation water (Bouaroudj et al., 2019). In addition, an increase in the soil EC may also be attributes to minerals load in the soil through irrigation water (Atta et al., 2023; Bouaroudj et al., 2019). The concentration of salts in wastewater can alter the osmotic potential between plants and soil, which can thereby affect the water and mineral balance of plants (Alturiqi et al., 2020; Bouaroudj et al., 2019; Soleimani et al., 2023).

The organic matter (OM) level in only TS3 was lower than in the other irrigated soils, while the highest OM level was found in TS4 (Table 3). Consistent with our findings, Travis et al. (2010) and Bouaroudj et al. (2019) have found that soil OM levels may be affected by the quality of irrigation water. The low OM level in irrigated soil may stem from insufficient OM load in soil through irrigation water (Tibhirine et al., 2025) and from an elevated breakdown of OM in soil through biochemical cycles (Ball et al., 2023; Doğan Demir & Sahin, 2020). However, the high level of OM in the soil may result from the discharge of OM in the soil through low-quality irrigation water (Nuñez et al., 2022) and from increased root deposit in the rhizosphere (Berg & Smalla, 2009; Dennis et al., 2010; Manegabe et al., 2025a). Soil OM significantly influences the mobility and availability of heavy metals in the soil for plant uptake (Khaskhoussy et al., 2022; Król et al., 2020; Li et al., 2021). In addition, OM can provide an optimal environment for microbial activities (Rahimi et al., 2021); and may monitor the soil pH due to the presence of basic functional groups, such as carboxylic, phenolic, amine, hydroxyl, and amide in biochemical molecules (Abegunrin et al., 2016; Doğan Demir & Sahin, 2020).

The concentrations of heavy metals in the rhizosphere of Swiss chard seedlings varied according to the type of water used in irrigation. In parallel with the relatively low level of heavy metals in TW, the concentrations of heavy metals were relatively low in TS1 (Table 3). The highest concentrations of Cr, Zn, and Pb were detected in TS4, whereas Cd exhibited elevated concentration in the soil irrigated with RW (TS2). Additionally, the highest concentrations of Co and As were detected in the soil exposed to AMD (TS3) (Table 3). The concentrations of almost heavy metals in irrigated soils, including the control was above permissible limits set for irrigated soils (Atta et al., 2023; Barakat et al., 2020). Consistent with our results, Soleimani et al. (2023) detected heavy metals (As, Cd, Pb, Cu, Fe, Zn, Cr, Mn, and Ni) in soils exposed to river water (RW), treated water effluent (TWE), and treated well water effluent (TWE). The tendency of heavy metal pollution increased in the order of WWF > RW > TWE. Hassan et al. (2024) and Victoria and Nnebini (2025) indicated that low-quality water reuse in agriculture may substantially affect the concentration of heavy metals in soils. Apart from low-quality irrigation water, previous publications indicate that airborne metals, rainfall (Angon et al., 2024; Manegabe et al., 2025a), and farming practices involving fertilizers and pesticides can increase the level of heavy metals in irrigated soils (Wan et al., 2024).

The concentrations of heavy metals in irrigated soil may be influenced by the level of heavy metals present in the irrigation (Ahmed et al., 2022), pH, and the OM load introduced to the soil by irrigation (Manegabe et al., 2025b). Many publications indicated that OM may regulate heavy metal concentrations in irrigated soils (Rahimi et al., 2021; Victoria & Nnebini, 2025; Yerli et al., 2025). Reports demonstrated that OM may chelate heavy metals, leading to their increase in the soil (Shabalala et al., 2022; Soleimani et al., 2023). This is largely explained by the presence of negative charges in the structure of OM that can bind cations through electrostatic phenomena (Yerli et al., 2025). However, the soil with low OM level may accumulates low levels of heavy metals due to low pH that increases the mobility and availability of heavy metals (Manegabe et al., 2025a). A comparison with previous findings indicated that the concentrations of Cr, Co, Zn, and As exceeded those detected in wastewater-irrigated soil in Iran (Soleimani et al., 2023) and Iraq (Tariq, 2021). However, the concentrations of Cd and Pb were lower than

those reported in irrigated soil in Ethiopia (Alturiqi et al., 2020).

4.2 Bacteria Community Structure in the Rhizosphere of Swiss Chard Seedlings

The physicochemical properties and heavy metal content in the soil brought about by irrigation water may alter soil bacterial abundance and diversity (Dang et al., 2019; Li et al., 2016; Obayomi et al., 2021). Many publications indicated that irrigation based on low quality water increased the level of OM in the soil, which account for nutrients sources for microbial community (Becerra-Castro et al., 2015; Liu et al., 2022; Obayomi et al., 2021), while heavy metals may severely affect bacterial community structure (Liu et al., 2022; Moulia et al., 2023; Zhang et al., 2008). In the present study, alpha diversity indices Chao1, Shannon, ACE, and Simpson, which are used to evaluate bacterial diversity, varied according to the type of water used in irrigation of Swiss chard seedlings (Fig. 1). However, the comparison between those indexes did not reveal significant differences ($p > 0.05$) between irrigated soils. Consistent with our findings, Mkhinini et al. (2020) and Krause et al. (2020) noted that irrigation water did not affect bacterial diversity in irrigated soils. Likewise, Xi et al. (2021) found that pollution by heavy metals (Cd, Cr, Cu, Pb, Zn, and Ni) in soils exposed to domestic and industrial treated wastewater did not decrease bacterial diversity and abundance. However, Zhang et al. (2008) conducted a study which reported that pollution by heavy metals (Pb, Hg, Cu, Cr, Cd) in wastewater-irrigated soil. They found that microbial diversity in the soil was seriously altered by heavy metals.

The bacterial diversity and abundance in irrigated soils reflect bacterial adaptation to changes brought about by in low-quality irrigation water in the soil (Chen et al., 2017; Krause et al., 2020; Obayomi et al., 2021). Indeed, OM load in low quality water irrigated soil may chelate toxic metals, which can potentially provide an optimal environment for the growth of bacteria (Manegabe et al., 2025a). In addition, OM may provide nutrients sources and may also regulate the soil pH which can then enhance bacterial multiplication in the soil (Guo et al., 2022; Obayomi et al., 2021). Many publications have reported that heavy metal pollution in irrigated soil can selected susceptible bacteria, which can potentially alter their diversity in irrigated soil (Joshi et al., 2023; Mathivanan et al., 2021). On the other hand, heavy metal pollution in irrigated soil may induce resistance/tolerance mechanisms in soil bacteria to withstand metal toxicity (Chen et al., 2017; Guo et al., 2022; Mathivanan et al., 2021). The tolerance/resistance of bacteria to metals in irrigated soil may result from mutations in bacterial genome and/or from the transfer of mobile genetic elements (MGEs) between exogenous bacteria in water and autochthonous bacteria soil (Götz & Smalla, 1997; Heuer & Smalla, 2007, 2012; Moulia et al., 2023; Soufi et al., 2025). The expression of acquired resistant/tolerant genes may result into metal efflux through bacterial membranes, metal sequestration, enzymatic detoxification, and complexation of metal into less toxic forms (Manegabe et al., 2017, 2025a; Ntabugi et al., 2020).

The relative abundance of bacteria in irrigated soil varied according to the quality of irrigation water (Cui et al., 2019; Moulia et al., 2023). In this study, the genera *Amaricoccus*, *Gemmatimonas*, *Microvirgas*, and S*phingomonas* were abundant in TS1, whereas *Lyzobacte*r and *RB41* predominated in TS2. Furthermore, *Blastococcus* and *Microlunatus* were abundant in TS3, while *Bacillus* and *Pir4 lineages* were dominant in TS4. Moreover, *Nocardioides*, *Solirubrobacte*r, and *Streptomyces* were abundant in TS5 (Fig. 2a). Around 44.4% of bacterial phyla were abundant in TS4. Moreover, *Armatimonadota*, *Gemmatimonadota*, *Myxococcota*, and *Proteobacteria* were dominant in TS1, while *Bacteroidota* and *Entotheonellaeta* were abundant in TS2. Furthermore, *Chloroflexi* was dominant in TS3, whereas *Chloroflexi*, *Firmicutes*, and *Methylomirabilota* were abundant in TS5 (Supplementary data, Table 2). Consistent with our results, Ibekwe et al. (2018) and Obayomi et al. (2021) showed a considerable increase in microbial abundance in low-quality irrigated soils. Cui et al. (2019) found that *Chloroflexi*, *Myxococcota*, and *Actinobacteria* were abundant in reclaimed water and piggery wastewater-treated soils. Moulia et al. (2023) analyzed microbial community structure in the rhizosphere of lettuce exposed to low-quality irrigation water. They found that *Proteobacteria*, *Chloroflexi*, *Planctomycetes*, *Actinobacteria*, and *Bacteroidetes* were dominant in wastewater irrigated soil, whereas *Firmicutes* and *Proteobacteria* were dominant in tap water-irrigated soil. Wei et al. (2017) Investigated on the diversity and abundance of bacteria in the soil

exposed to reclaimed water. The results showed that *Proteobacteria*, *Gemmatimonadetes*, and *Bacteroidetes* were abundance in the soils. Many publications (Chen et al., 2017; Dang et al., 2019; Obayomi et al., 2021) reported that the most abundant bacteria in wastewater irrigated soils were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Planctomycetes*. Guo et al. (2022) investigated bacterial abundance in the soil exposed to river water. They found that the abundant bacteria were *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*. The abundance of bacterial in irrigated soil may be attributed to the level OM and nutrients in the soil (Liu et al., 2022; Moulia et al., 2023). Some reports indicated OM provide essential nutrient and may also stabilize the pH at an optimal level which can facilitated the bacterial multiplication, affecting their abundance in the soil (El-Khateeb et al., 2023; Espira et al., 2024; Ibekwe et al., 2018; Obayomi et al., 2021; Soufi et al., 2025).

5 Conclusion

This study revealed that the type of water used in the irrigation of seedlings affected bacterial community structure and heavy metal contents in the rhizosphere of Swiss chard seedlings. Consequently, nearly three-quarters of the heavy metals were detected at low concentrations in the soil exposed to tap water, while the highest concentrations of individual heavy metals in soils fluctuated according to the quality of irrigation water. Similarly, bacterial diversity and abundance in soils varied according to the type of irrigation water. The RDA findings indicated that the bacterial community structure in the rhizosphere of Swiss chard seedlings was affected by abiotic factors, including heavy metals, pH, electrical conductivity, and organic matter. Heavy metal pollution in low-quality irrigated soil may induce the horizontal gene transfer between aquatic bacteria and soil bacteria, affecting bacterial adaptation and diversity, while organic matter and nutrient influx from irrigation may chelate heavy metals and may also supply essential nutrients to bacteria, potentially promoting their multiplication. This study opens up new frontiers in expanding metagenomics studies in the rhizosphere of seedlings. Further investigations may explore alterations in the bacterial genome induced by heavy metal in the rhizosphere of Swiss chard seedlings for a deeper understanding of the role of rhizosphere bacteria in bioremediation and the spread of antibiotic resistance genes that could potentially pose a risk to human and plant health.

Author Contribution 1. Bahati J. Manegabe: Conceived the project, wrote the manuscript and did the experiment.
2. Titus AM. Msagati: supervised the research, edited the manuscript, and assessed dada accuracy.
3. Marie-Médiatrice Kikongo Ntabugi: Provided the methodology and did statistical analysis.
4. Rian Pierneef: did bioinformatics analysis of 16S rRNA sequences.
5. Johannes P. Maree: Analyzed heavy metals in the samples and assessed data accuracy.
6. Karin De Bryun: edited the manuscript and verified manuscript accuracy.
7. Maropeng V. Raletsena: edited the manuscript and verified manuscript accuracy.

Funding Open access funding provided by University of South Africa.

Data Availability Available data for this study are presented in supplementary materials.

Declarations

Ethical Approval Not applied (N/A).

Consent for Publication Not applied (N/A).

Conflict of interest Authors declare no conflict of interest.

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