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Irrigation of maize with treated water applying bacteria and agave extract as plant biostimulants: production, N status and heavy metals

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Research Article

Keywords: agricultural water, PGPB, saponins, WWTP effluent, NBI, trace elements

Posted Date: October 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10991016/v1

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Additional Declarations: No competing interests reported.

Abstract

Water scarcity drives the search for alternative sources for agricultural irrigation, such as treated wastewater (TWW). This study evaluated the effects of TWW, *Agave lechuguilla* Torrey extract (ALE), and plant growth-promoting bacteria (PGPB) on maize (*Zea mays* L.) production, ear quality, nitrogen status, and heavy metal accumulation. Five treatments were evaluated under a randomized complete block design with five replications: TWW combined with two ALE doses (0 and 0.9 mL m^{-2}) and two PGPB levels (0 and 7 mL L^{-1} of 2×10^8 and 1×10^9 CFU mL^{-1} liquid suspension of *Pseudomonas fluorescens* and *Raoultella terrigena*, respectively), plus a potable water control (PW). Growth, yield, ear quality, nitrogen status (chlorophyll, flavonoids, anthocyanins, and Nitrogen Balance Index), and kernel heavy metal concentrations were determined. TWW irrigation produced similar effects to PW on maize growth, yield, ear quality, and nitrogen status. Orthogonal contrast analysis revealed that ALE application increased fresh ear weight and ear length by 22% and 8%, respectively; PGPB did not significantly affect any variable. Principal component analysis confirmed the similarity between TWW and treatments and identified ALE as the most promising complementary treatment. Kernels irrigated with TWW accumulated more As, Cr, Cu, Fe, Mn, and Mo; As, Cr, and Cu exceeded maximum permissible limits. TWW can be used as an alternative irrigation source for maize without negatively affecting productivity or quality. However, additional measures are required to reduce heavy metal accumulation in edible plant tissues to ensure food safety and sustainable agricultural reuse of TWW.

1 Introduction

Water scarcity is one of the most critical global challenges of the 21st century (Oniyena & Sam, 2025). It is caused by the unsustainable exploitation of water resources to meet the demands of the growing world population, which is projected to reach 8.6 billion by 2030 and 9.8 billion by 2050 (UN, 2025). The primary cause of this increasing demand is the growing need for food, which has led to the intensification of agriculture and, consequently, to a greater demand for water resources. This situation has driven the search for alternative strategies and uses of water, such as the utilization of treated wastewater for agricultural irrigation (Al Hamedi et al., 2023).

The use of treated wastewater for agricultural irrigation reduces freshwater demand and minimizes contamination of surface and groundwater resources. In addition, treated wastewater contains nutrients and organic matter that, when properly managed, can improve soil fertility and crop productivity (Ray et al., 2025). However, treated wastewater may also contain substances that can negatively affect crop production due to changes in soil quality, including sodium, salts, carbonates, and other compounds, as well as heavy metals and metalloids that may be taken up by plants and pose risks to consumer health (Rodríguez et al., 2009; Al Shali et al., 2024), potentially causing organ diseases, neurological disorders, and cancer (Robles, 2024).

Ensuring the availability and sustainable management es a key United Nations goal for 2030 (UN, 2015), where agriculture is the main economic activity and plays a key role in poverty reduction. However, it is essential to develop and implement strategies that ensure efficient water use while maintaining agricultural productivity, environmental integrity, and human health (Ray et al., 2025). Therefore, it is necessary to explore the use of treated wastewater as an alternative resource for agricultural irrigation, considering all its potential implications (Al Hamedi et al., 2023), and to generate scientific information that supports its safe use.

In addition to water management, agricultural production can be improved through the use of biostimulants, which are substances or microorganisms that enhance natural biological processes. Agricultural biostimulants have considerable potential to improve crop productivity and contribute to the sustainability of agroecosystems. They can enhance nutrient uptake and increase plant tolerance to stress conditions. However, substantial variability in their

effectiveness and limited knowledge regarding their performance under field conditions have been reported (Sible et al., 2021; López et al., 2026).

Among the wide diversity of biostimulants that may mitigate the adverse effects of treated wastewater on plants, such as salinity, alkalinity, and metal toxicity, is the extract of *Agave lechuguilla* Torrey (ALE). This substance is produced through the secondary metabolism of plants and contains steroids, terpenoid glycosides (Porte et al., 2022), and between 0.02 and 1.69% saponins (Rosas et al., 2005). Previous studies have reported its surfactant properties for heavy metal removal (Mulliga, 2021), as well as positive effects on root growth in the grass *Lolium perenne* (Duran et al., 2022) and on maize seed germination (Sharma et al., 2023).

Likewise, some plant growth-promoting bacteria (PGPB) are capable of tolerating dry and saline environments while enhancing various plant processes, including germination, seedling vigor, root development, shoot growth, and chlorophyll accumulation, among others (Sarkar et al., 2018). Bacterial strains belonging to the genera *Pseudomonas* and *Raoultella* have been identified in the rhizosphere of maize crops and characterized by their ability to solubilize various nutrients and protect plants against drought stress (Ikhwan et al., 2022; Gen et al., 2024).

Therefore, due to the environmental impacts generated by agricultural activities in recent years, conventional agriculture is increasingly shifting toward more sustainable and environmentally responsible strategies worldwide (Boix and de Vente et al., 2022), including the use of treated wastewater, biostimulants, and plant growth-promoting bacteria.

One of the most important crops worldwide is maize (*Zea mays* L.), which is primarily consumed as grain by humans, with an annual per capita consumption of approximately 18.5 kg, and to a lesser extent in its immature form as fresh corn (corn ear).

Accordingly, the objective of the present study was to generate basic information regarding the sustainable use of treated wastewater in agriculture by evaluating its effects on yield, ear quality, and nitrogen status in maize irrigated with treated wastewater and supplemented with *Agave lechuguilla* Torrey extract and inoculation with plant growth-promoting bacteria of the genera *Pseudomonas and Raoultella*. Additionally, the accumulation of trace elements in maize ears irrigated with treated wastewater was determined.

2 Materials and Methods

2.1 Study Site

The experiment was conducted at the facilities of the Food Self-Production Training Center of the governmental agency *Desarrollo Integral de la Familia* (State DIF), located at 22°07'34" N and 101°00'01" W within Tangamanga I Park in San Luis Potosí City, Mexico.

2.2 Water Analysis

The treated wastewater used in this study was previously characterized to determine its physicochemical properties and assess whether its composition could negatively affect maize production. Three samples of the effluent from a wastewater treatment plant (WWTP) located in San Luis Potosí City were collected on different days during the week prior to the beginning of the experiment. The WWTP treats municipal wastewater using the activated sludge process.

In the laboratory, the following parameters were determined for each sample: electrical conductivity (EC, dS m^{-1} at 25°C), pH, nitrate concentration (ppm), phosphate concentration (ppm), biochemical oxygen demand (BOD_5, mg O_2

L^{-1}), calcium (ppm), magnesium (ppm), sodium (ppm), potassium (ppm), carbonates (ppm), bicarbonates (ppm), chlorides (ppm), sulfates (ppm), hardness (mg L^{-1}), sodium adsorption ratio (SAR, meq L^{-1}), and residual sodium carbonate (RSC, meq L^{-1}). The values obtained from the three samples were average (Table 1).

To compare the variability and similarity of treated wastewater composition with that of water commonly used for agricultural irrigation, as well as to compare their effects on maize yield, ear quality, and crop nitrogen status, three samples of potable water supplied by the municipal water distribution network were also collected and analyzed for the same parameters described above (Table 1).

Table 1
Physicochemical properties of potable water and treated wastewater used for maize irrigation.

Property	Unit	Potable water	Treated wastewater
EC	dS m^{-1} at 25°C	0.442	1.148
pH	—	7.1	7
Nitrates	ppm	33	40
Phosphates	ppm	0	1.18
BOD_5	mg O_2 L^{-1}	2.3	32
Calcium	ppm	64	22
Magnesium	ppm	18.3	24.4
Sodium	ppm	46	23
Potassium	ppm	39.1	82.1
Carbonates	ppm	0	0
Bicarbonates	ppm	305	256.2
Chlorides	ppm	71	35.5
Sulfates	ppm	33	48
Hardness	mg L^{-1}	23.53	15
SAR	meq L^{-1}	1.3	0.8
RSC	meq L^{-1}	0.3	1.1

EC = Electrical Conductivity. BOD_5 = Biochemical Oxygen Demand. SAR = Sodium Adsorption Ratio. RSC = Residual Sodium Carbonate

For the analysis of heavy metals present in the water, an additional sample from each water source was collected. In the laboratory, the concentrations (ppm) of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), titanium (Ti), vanadium (V), and zinc (Zn) were determined using optical emission spectroscopy (OES).

The results indicated that neither water source exceeded the maximum permissible limits (MPLs) established for agricultural irrigation use according to the Mexican Official Standard NOM-001-SEMARNAT-2021 (SEMARNAT, 2022) (Table 2).

Table 2
Heavy metal concentrations (mg L^{-1}) in potable water and treated wastewater used for maize irrigation.

Type	As	Cd	Cr	Cu	Fe	Li	Mg	Mn	Mo	Ni	Pb	Ti	V	Zn
PW	≤ 0.03	≤ 0.06	≤ 0.06	≤ 0.08	≤ 0.18	0.12	0.64	≤ 0.07	≤ 0.05	≤ 0.07	≤ 0.05	≤ 0.07	≤ 0.07	≤ 0.10
TWW	≤ 0.03	≤ 0.06	≤ 0.06	≤ 0.08	≤ 0.18	0.10	3.54	≤ 0.07	≤ 0.05	≤ 0.07	≤ 0.05	≤ 0.07	≤ 0.07	≤ 0.10
MPL	0.2	0.2	1	6	NS	NS	NS	NS	NS	4	0.4	NS	NS	20

PW = Potable water. TWW = Treated wastewater. MPL = Maximum permissible limit according to NOM-001-SEMARNAT-2021. NS = Not specified.

2.3 Experimental Unit

The experimental units consisted of 40 L plastic pots (0.1 m^2 surface area and 0.4 m height). The maize variety used was A7573 from Asgrow, a hybrid developed for fresh ear corn production, characterized by an intermediate growth cycle, white grain color, a flowering period of 65–70 days, and physiological maturity at 170–180 days. Three maize seeds were sown in each pot.

The substrate consisted of a mixture of peat moss, poultry manure, alluvial soil, and sand in a ratio of 1:0.5:2:2 (v/v), respectively. Its physical and chemical characteristics were as follows: total porosity, 49%; air-filled porosity, 8%; water-holding capacity, 43%; pH, 7.12; electrical conductivity, 0.56 dS m^{-1}; organic matter, 6.9%; cation exchange capacity, 32.1 cmol(+) kg^{-1}; exchangeable Na, 2.28 cmol (+) kg^{-1}; and chloride concentration, 6.1 meq L^{-1}.

2.4 Treatments and Experimental Design

Five treatments were evaluated for maize irrigation using treated wastewater, resulting from the combination of two studied factors (*Agave lechuguilla* Torrey extract [ALE] and plant growth-promoting bacteria [PGPB]), plus a control treatment irrigated with potable water (Table 3). The experiment was conducted using a randomized complete block design with five replications.

Table 3
Irrigation treatments with treated wastewater supplemented with biostimulants *(Agave lechuguilla* Torrey extract [ALT] and plant growth-promoting bacteria [PGPB]).

Treatment	Type of water	ALT	PGPB*
		mL m^{-2}	mL L^{-1}
1	Treated wastewater	0	0
2	Treated wastewater	0	7
3	Treated wastewater	0.9	0
4	Treated wastewater	0.9	7
5	Potable water	0	0

*Concentration of plant growth-promoting bacteria (PGPB) = 2×10^8 and 1×10^9 CFU mL^{-1} for *Pseudomonas fluorescens* and *Raoultella terrigena*, respectively

The biostimulant product was a commercial formulation supplied by Biotika® and certified by OMRI. It was used as a source of steroidal saponins and consisted of a liquid formulation containing 98% *Agave lechuguilla* Torrey extract and

2% diluents. The saponin extract was applied at 25, 44, and 56 days after sowing (DAS) at rates of 0 and 0.9 mL m^{-2}.

The plant growth-promoting bacteria (PGPB) consisted of a mixture of *Pseudomonas fluorescens* and *Raoultella terrigena* provided by the Microbiology Laboratory of the Benemérita Universidad Autónoma de Puebla. The bacterial inoculum was supplied as a 1 L aqueous suspension with concentrations of 2×10^8 and 1×10^9 CFU mL^{-1}, respectively. The bacterial suspension was applied at 23, 46, and 58 DAS at rates of 0 and 7 mL of the concentrate per pot.

2.5 Study Variables

The following production variables were determined at 120 days after sowing using five plants per replication and treatment:

Plant height (cm): measured from the substrate surface to the plant apex using a measuring tape graduated in 1-cm increments. Stem diameter (mm): measured at the widest section of the stem using a digital caliper. Leaf area per plant (cm^2): calculated for each leaf as length (cm) × width (cm) × 0.75 (maize correction factor) and then summed across all leaves per plant. Plant dry weight (g): plants were cut at the substrate level and ears were separated. Plant material was dried in a forced-air oven (ELVEC) at 65°C until constant weight and weighed using a digital balance (Ohaus).

The ear quality variables were: Fresh ear weight (g): ears were harvested and immediately weighed, both with and without husks, using a digital balance. Ear length and ear width (cm): the husks and silks were removed, the ear was placed on a flat and stable surface, and length and transverse measurements were taken using a measuring tape with 1-mm graduations. Soluble solids content (°Brix): fresh kernels were collected from the middle section of each ear, crushed using forceps, and the extracted juice was analyzed with a handheld refractometer (ATAGO®).

The nitrogen status variables included: Nitrogen Balance Index (NBI), Chlorophyll, Flavonoids, and Anthocyanins. These variables were measured using a Dualex meter (METOS).

Heavy metals in maize kernels were determined only in the treated wastewater and potable water treatments. Maize kernels were dried in a forced-air oven at 65°C until constant weight, ground, and a 0.25-g subsample was digested with concentrated nitric acid using a microwave digestion system (Mars Model 6). Heavy metal concentrations were subsequently determined using an optical emission spectrometer (Optima 8300).

2.6 Data Analysis

Data were subjected to analysis of variance (ANOVA; $p < 0.05$), followed by orthogonal contrast analyses. Three orthogonal contrasts were performed: Contrast 1 (C1) compared irrigation with treated wastewater versus potable water; Contrast 2 (C2) compared the *Agave lechuguilla* Torrey extract (ALE) rates; and Contrast 3 (C3) compared the PGPB rates. Table 4 presents the coefficients and hypotheses associated with orthogonal contrasts.

Statistical analyses were performed using Statistical Analysis Software (SAS) version 22 (IBM, 2013). To visualize differences among the treated wastewater and potable water treatments based on the evaluated plant variables, a principal component analysis (PCA) and its graphical representation were conducted using RStudio (version 2025.05.0 + 496) with the FactoMineR (v. 3.5.3) and factoextra (v. 3.5.3) packages.

Table 4
Orthogonal contrasts, descriptions, and hypotheses.

Contrast	T1	T2	T3	T4	T5	Description	Hypothesis
C1	1	0	0	0	-1	Treated wastewater vs. potable water	Ho: T1-T5 = 0 Ha: T1-T5 ≠ 0
C2	1	1	-1	-1	0	Without ALT vs. with ALT	Ho: T1 + T2-T3 + T4=0 Ha: T1 + T2-T3 + T4≠0
C3	1	-1	1	-1	0	Without PGPB vs. with PGPB	Ho: T1 + T3-T2 + T4=0 Ha: T1 + T3-T2 + T4≠0

3 Results and Discussion

3.1 Orthogonal Contrast 1: Comparison of Maize Irrigation with Treated Wastewater versus Potable Water

The results of Orthogonal Contrast 1 analysis indicated significant differences in fresh ear weight, with the potable water treatment showing higher values than the treated wastewater treatment (PW > TW), and in total plant dry weight (excluding ears), where the treated wastewater treatment showed higher values than the potable water treatment (TW > PW) ($p \leq 0.05$). No significant differences were observed for the remaining growth variables or for the plant nitrogen status variables ($p \leq 0.05$) (Tables 5 and 6).

Table 5
Effects of treated wastewater and potable water irrigation on maize growth and production variables.

Treatment	Fresh ear weight with husks (g)	Fresh ear weight without husks (g)	PTDW (g)	Plant height (cm)	Stem diameter (cm)	Leaf area (cm²)
TWW	206.4 ± 23.6a	79.2 ± 9.8a	66.40 ± 10.0b	117 ± 6.1a	2.2 ± 0.14a	6116 ± 934a
PW	198.5 ± 14.9a	73.9 ± 7.5b	70.43 ± 7.6a	116 ± 12.4a	2.4 ± 0.35a	6354 ± 865a

TWW = treated wastewater; PW = potable water; PTDW = plant total dry weight (excluding ears). Values are means ± standard error. Different letters indicate significant differences ($p < 0.05$).

Table 6
Effects of irrigation with treated wastewater and potable water on nitrogen status variables in maize plants and ears.

Treatment	Chlorophyll	NBI	Flavonoids	Anthocyanins	Ear length (cm)	Ear width (cm)	Grain Brix value
TWW	51.5 ± 4.6a	57.2 ± 4.2a	0.9 ± 0.12a	0.10 ± 0.04a	19.0 ± 0.7a	4.8 ± 0.4a	10.5 ± 0.8a
PW	52.8 ± 2.3a	53.1 ± 5.8a	1.0 ± 0.16a	0.11 ± 0.03a	19.5 ± 0.5a	5.0 ± 0.5a	10.4 ± 0.5a

TWW = treated wastewater; PW = potable water. Values are means ± standard error. Different letters indicate significant differences ($p < 0.05$).

These results differ from those reported in previous studies since, in general, crops irrigated with treated wastewater tend to exhibit increased yield, improved quality, and enhanced nutritional status, which has been attributed to the higher content of organic matter and essential nutrients present in the water (Hanjra et al., 2012; Chojnacka et al., 2020). However, although the treated wastewater used in this study contained higher concentrations of NO_3-N and organic matter (Table 1), the difference was not sufficient to affect the nutritional status or nitrogen metabolism of the plants, as evidenced by the lack of significant differences in chlorophyll concentration and NBI values (Table 5).

Velasco et al. (2023) found that maize irrigated with treated wastewater showed significant increases in plant height (142%), stem diameter (123%), root dry weight (244%), leaf dry weight (227%), and stem dry weight (291%). They also reported that treated wastewater significantly increased nitrogen concentrations in roots, leaves, and stems, with values more than double those observed under potable water irrigation. Similarly, Abunama et al. (2014) reported positive effects on maize productivity and plant development and concluded that treated wastewater can be safely used for agricultural irrigation in accordance with the guidelines and regulations established by the World Health Organization (WHO) for the reuse of treated wastewater in agriculture. Likewise, Saleh et al., (2025) reported that maize quality was not significantly affected by irrigation with treated wastewater.

The similarity observed between treated wastewater and potable water irrigation is important because maize production, yield, and quality were not compromised, at least for the variables evaluated in this study. Therefore, the use of treated wastewater may represent a viable alternative for agricultural irrigation. One of the most relevant benefits of using treated wastewater in agriculture is the reduction in pressure on freshwater resources (Jaramillo & Restrepo, 2018), which, in the case of maize cultivation, could represent savings of between 5,000 and 8,000 $m^3\ ha^{-1}$ per growing cycle (Sáez et al., 2024). Another associated benefit is the reduction in energy consumption required for water pumping, particularly from deep wells. Furthermore, due to the contribution of dissolved nutrients, fertilizer savings could be substantial. In the present study, treated wastewater supplied higher concentrations of nitrate (21%), magnesium (33%), and potassium (110%) compared with potable water.

3.2 Orthogonal Contrast 2: Effect of *Agave lechuguilla* Torrey Extract (ALE)

The results of Orthogonal Contrast 2 indicated that the application of 0.9 mL m^{-2} of ALE significantly increased fresh ear weight and ear length. Increases of 22% and 8%, respectively, were observed compared with the treatment without ALE application ($p \leq 0.05$) (Tables 7 and 8).

Table 7
Effect of *Agave lechuguilla* Torrey extract (ALT) on growth and production variables of maize plants and ears irrigated with treated wastewater and potable water.

ALT treatment	Fresh ear weight with husks (g)	Fresh ear weight without husks (g)	PTDW (g)	Plant height (cm)	Stem diameter (cm)	Leaf area (cm^2)
0 mg L^{-1}	212.6 ± 31.8a	66.4 ± 14.6b	62.8 ± 8.6a	117.1 ± 8.7a	2.25 ± 0.09a	6320 ± 1616a
0.9 mg L^{-1}	228.5 ± 29.7a	81.3 ± 20.1a	60.7 ± 7.8a	118.5 ± 13.4a	2.37 ± 0.15a	6698 ± 1491a

PTDW = plant total dry weight (excluding ears). Values are means ± standard error. Different letters indicate significant differences ($p < 0.05$).

Table 8
Effect of *Agave lechuguilla* Torrey extract (ALT) on nitrogen status and ear quality variables of maize irrigated with treated wastewater and potable water.

ALT treatment	Chlorophyll	NBI	Flavonoids	Anthocyanins	Ear length (cm)	Ear diameter (cm)	°Brix
0 mg L^{-1}	51.1 ± 3.6a	54.8 ± 6.3a	0.9 ± 0.1a	0.10 ± 0.02a	19.1 ± 0.9b	4.8 ± 0.4a	10.2 ± 0.68a
0.9 mg L^{-1}	49.1 ± 4.2a	49.7 ± 6.3a	1.0 ± 0.1a	0.10 ± 0.01a	21.6 ± 1.1a	4.8 ± 0.5a	10.1 ± 0.47a

NBI = Nitrogen Balance Index. Values are means ± standard error. Different letters indicate significant differences ($p < 0.05$).

The stimulatory effect on ear length confirms one of the functions attributed to saponins, namely their ability to promote the growth of fruits or plant organs (Makkar et al., 2007). However, other proposed functions, such as enhancing overall plant growth and quality, particularly soluble solids content (°Brix), could not be confirmed in the present study. Saponins have shown both positive and negative effects on crops. In this regard, steroidal saponins have exhibited phytotoxic effects and, therefore, may be considered potential herbicides (Duran *et al*., 2022).

The application of ALE did not significantly affect nitrogen status variables, including chlorophyll concentration and NBI. One study reported that the application of saponins at a 5% concentration in soybean significantly affected chlorophyll and carotenoid contents while improving the antioxidant system, osmolyte metabolism, and significantly reducing sodium ion accumulation (Soliman et al., 2020). Nevertheless, studies evaluating the effects of steroidal saponins on plant nitrogen status remain scarce.

Further research is needed to confirm the effects of saponins on maize yield and quality, as well as to evaluate the effects of different types of saponins, application rates, application frequencies, timing of application, and their interactions with soil properties and other agricultural inputs.

3.3 Orthogonal Contrast 3: Effect of Plant Growth-Promoting Bacteria (PGPB) Inoculation (*Pseudomonas fluorescens* and *Raoultella terrigena*)

The results of Orthogonal Contrast 3 indicated that inoculation with PGPB did not significantly affect growth variables or plant nitrogen status in maize irrigated with treated wastewater ($p \leq 0.05$) (Tables 9 and 10).

Table 9
Effects of PGPB inoculation on growth and production variables of maize plants and ears irrigated with treated wastewater and potable water.

PGPB treatment	Fresh ear weight with husks (g)	Fresh ear weight without husks (g)	PTDW (g)	Plant height (cm)	Stem diameter (cm)	Leaf area (cm^2)
0 mL L^{-1}	213.6 ± 37.0a	80.6 ± 20.8a	61.5 ± 11.5a	119.4 ± 10.21a	2.2 ± 0.13a	5461 ± 1469b
7 mL L^{-1}	219.1 ± 24.0a	77.0 ± 14.3a	62.0 ± 4.8a	119.3 ± 11.94a	2.3 ± 0.10a	6460 ± 1638a

PGPB = plant growth-promoting bacteria; PTDW = plant total dry weight (excluding ears). Values are means ± standard error. Different letters indicate significant differences ($p < 0.05$).

Table 10
Effects of PGPB inoculation on nitrogen status variables in maize plants and ears irrigated with treated wastewater and potable water.

PGPB treatment	Chlorophyll	NBI	Flavonoids	Anthocyanins	Ear length (cm)	Ear width (cm)	°Brix
0 mL L^{-1}	49.5 ± 4.1a	51.4 ± 3.1a	0.9 ± 0.1a	0.10 ± 0.025a	19.7 ± 0.7a	4.8 ± 0.4a	10.1 ± 0.68a
7 mL L^{-1}	50.8 ± 3.7a	51.7 ± 9.5a	0.9 ± 0.1a	0.10 ± 0.017a	20.6 ± 1.3a	4.9 ± 0.3a	10.3 ± 0.45a

NBI = Nitrogen Balance Index. Values are means ± standard error. Different letters indicate significant differences ($p < 0.05$).

High competition between PGPB and the native soil microbiome, insufficient inoculum dosage, or various types of stress (water, salinity, or temperature stress) may explain the lack of a significant response to bacterial inoculation observed in this study (Hassain et al., 2023). However, considering the results obtained, a positive effect could potentially be achieved by increasing the PGPB dose applied (Table 10). Papin et al. (2025) reported that, despite the rapid development of microbial inoculants, their effectiveness still lacks robustness, partly due to the limited understanding of the factors influencing their establishment in the soil. Nevertheless, future studies should investigate whether these bacterial strains can express their full potential as PGPB in maize, as has been demonstrated in crops such as *Medicago lupulina*, where *Raoultella terrigena* considerably increased root growth (Sarron et al., 2018). *Raoultella* has been reported to possess the ability to solubilize phosphate and potassium, produce indole-3-acetic acid (IAA), fix atmospheric nitrogen, and produce siderophores (Lucumi et al., 2025).

Likewise, recurrent applications of *Pseudomonas fluorescens* have been reported to produce greater changes in the resident bacterial community than a single inoculation event, which likely contributed to a 47.4% increase in maize shoot dry weight (Papin et al., 2025). Another study demonstrated a synergistic effect when *P. fluorescens*, wheat residues, and organic extracts were applied together, significantly reducing soil electrical conductivity and pH while increasing plant height and maize dry weight (Kareem et al., 2025).

3.4 Principal Component Analysis of Treated Wastewater and Potable Water Treatments

The principal component analysis (PCA) of the different treatments revealed a heterogeneous spatial distribution of the samples. However, the centroids (representing the average position of the projected samples) of treatments 2 (treated wastewater + 0 mL m^{-2} saponins + 7 mL L^{-1} PGPB), 4 (treated wastewater + 0.9 mL m^{-2} saponins + 7 mL L^{-1} PGPB), and 5 (potable water + 0 mL m^{-2} saponins + 0 mL L^{-1} PGPB) formed a closely clustered group, indicating similar effects on the response variables. In contrast, these treatments differed slightly from treatments 1 (treated wastewater + 0 mL m^{-2} saponins + 0 mL L^{-1} PGPB) and 3 (treated wastewater + 0.9 mL m^{-2} saponins + 0 mL L^{-1} PGPB), as well as from each other.

These results indicate that irrigation with treated wastewater produced effects similar to those obtained with potable water irrigation for the evaluated response variables, thereby reinforcing the findings obtained from Orthogonal Contrast 1. Treatment 3 (treated wastewater + 0.9 mL m^{-2} saponins + 0 mL L^{-1} PGPB) exhibited a slightly different spatial distribution compared with the remaining treatments and based on the results of Orthogonal Contrast 2, appeared to be the best-performing treatment.

Dimensions 1 and 2 explained 39.53% of the total variation in the dataset. Dimension 1 accounted for 22.64% of the variation (eigenvalue = 2.94) and was primarily associated with NBI, flavonoids, fresh ear weight without husks, and soluble solids content (°Brix) ($p < 0.05$). Dimension 2 explained 16.89% of the variation (eigenvalue = 2.19) and was mainly associated with ear length, stem diameter, and chlorophyll concentration ($p < 0.05$) (Fig. 1).

3.5 Heavy Metals in Maize Kernels Irrigated with Treated Wastewater

The results of heavy metal concentrations in maize kernels are presented in Table 11. Of the fourteen metals evaluated, As, Cr, Cu, Fe, Mn, Mo, and Ti concentrations were higher in the treated wastewater treatment than in the potable water treatment. In contrast, Mg and Zn concentrations were higher in potable water treatment. For Cd, Ni, Pb, and V, concentrations were identical in both treatments.

Table 11
Heavy metal concentrations in maize kernels irrigated with potable water and treated wastewater (mg kg^{-1}).

IR	As	Cd	Cr	Cu	Fe	Mg	Mn	Mo	Ni	Pb	Ti	V	Zn
PW	0.6	< 0.06	< 0.06	1.2	< 0.18	804	2.2	0.4	< 0.07	< 0.05	< 0.07	< 0.07	14.3
TWW	1.2	< 0.06	2.0	21.5	7.0	709	2.3	1.2	< 0.07	< 0.05	< 0.07	< 0.07	10.5
MPL	0.2*	0.2	1	20	NS	NS	500	NS	NS	0.43	NS	NS	50

IR = Irrigation water; PW = Potable water; TW = Treated wastewater; MPL = Maximum permissible limit (European Union, 2006); NS = Not specified in the regulation; * Safe limit (World Health Organization, 2001).

Arsenic (As), chromium (Cr), and copper (Cu) were detected in maize kernels at concentrations exceeding the maximum permissible limits (MPLs) in the treatment irrigated with treated wastewater. Therefore, special attention should be given to these elements because of the potential risks they pose to human health.

Specifically, As is a toxic element for humans because it affects the integumentary, cardiovascular, immune, hepatic, renal, and respiratory systems, influences embryonic development, and possesses significant carcinogenic properties (Rodríguez-Padilla, 2021). The As concentration in maize kernels exceeded the MPLs under both irrigation treatments, reaching values three times higher than the limit under potable water irrigation and six times higher under treated wastewater irrigation (Table 11). Pais and Benton (2000) reported that As has a moderate bioaccumulation index in plants and would not generally be expected to accumulate through the food chain; therefore, this result is unusual in maize plants. Herrera et al. (2017) reported that As accumulated in maize tends to be stored primarily in the roots, reducing its concentration in aerial plant tissues according to the order root > leaf > grain. Likewise, Xu et al., (2023) reported that As can bind to hydroxyl groups in the outer epidermal cell walls of roots, thereby reducing its translocation.

Chromium (Cr) generally occurs in foods in its trivalent form and has beneficial effects related to glucose regulation, metabolic syndrome, polycystic ovary syndrome, and elevated cholesterol levels, among others (NIH, 2025). However, excessive intake may reduce iron absorption, cause mild anemia, and affect liver and kidney function (ATSDR, 2025). In the present study, the MPL for Cr was exceeded in maize kernels irrigated with treated wastewater, where a concentration of 2 ppm was detected (Table 11). Pais and Benton (2000) reported that Cr has a moderate bioaccumulation index in plants. Asgaris and Cornelis (2015) found no differences in soil Cr concentrations between

maize irrigated with freshwater and treated wastewater; however, they observed relatively higher Cr uptake in maize, representing a potential health risk. Adiloğlu and Göker (2021) concluded that maize is a Cr(VI)-accumulating species and may be used for the phytoremediation of soils contaminated with this element.

Copper (Cu), on the other hand, provides important benefits to human health and is essential for red blood cell formation, nervous system function, bone health, immune system performance, and energy production. However, excessive consumption may cause abdominal pain, nausea, vomiting, and diarrhea, while long-term exposure may lead to liver and kidney damage (NIH, 2025). The MPL for Cu in maize kernels was exceeded in the treated wastewater treatment, where its concentration was approximately twenty times greater than that observed under potable water irrigation (Table 11). Similar results were reported by Carner (2024), who found that maize irrigated with treated wastewater accumulated more Cu in silage than maize irrigated with potable water, although the concentrations did not exceed the FAO/WHO limit. Pais and Benton (2000) reported that Cu has a high bioaccumulation index in plants and, consequently, high mobility within the food chain.

The bioaccumulation of As, Cr, and Cu in maize kernels irrigated with treated wastewater, despite concentrations in the irrigation water being below the detection limits of the analytical equipment (As ≤ 0.03, Cr ≤ 0.06, Cu ≤ 0.08 ppm; Table 2), may be explained by three possible causes:

1. The presence of these elements in treated wastewater at concentrations just below the detection limit, yet sufficiently high to allow absorption and translocation into the maize ear.
2. The chemical speciation of these metals, which may have increased their bioavailability in treated wastewater. As may have been present as arsenate [As(V)] and arsenite [As(III)], which are its most bioavailable forms (Megharg, 2002). Suriyagoda et al. (2018) reported that microorganisms can transform As species from As(V) to As(III), and subsequently to monomethylarsonic acid (MMA; $CH_3AsO(OH)_2$) and dimethylarsinic acid (DMA; $(CH_3)_2AsOOH$), which can be absorbed through the silicon transporter Lsi1. Chromium in the Cr(VI) form is more readily absorbed by plants than Cr(III) because of its greater water solubility and higher transmembrane transport efficiency (Aharchaou et al., 2017). Pais and Benton (2000) indicated that Cu is preferentially absorbed in the Cu^{2+} form.
3. The involvement of transporters of different natures that may have facilitated the uptake and translocation of these elements. Plants utilize high-affinity phosphate transporters that also facilitate As uptake. Because the structure of As(V) is similar to that of phosphate, As competes for the same sorption sites in plant roots (Su et al., 2025). Phosphate addition generally has two consequences: (i) greater downward displacement of As, resulting in increased leaching from the upper soil layers, and (ii) increased availability of As in the soil solution (Abedi and Mojiri, 2020). Pigna et al. (2010) reported that As toxicity in crops may be exacerbated when As contamination coexists with low phosphorus availability. Regarding Cr, Ding et al. (2019) reported that Cr(VI) uptake is an active process dependent on phosphate or sulfate carriers because of structural similarities among these ions. In the case of Cu, transport across the plasma membrane occurs through channels or transport proteins (Hopkins, 1995). Specifically, Cu^{2+} may be transported through ZIP-type transporters (Pilon, 2011). Within the xylem, Cu may be transported by HMA5, a member of the P-type ATPase family that is also involved in the transport of heavy metals such as Fe^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} (Palmer and Guerinot, 2009).

Given this issue, several strategies should be considered to reduce and significantly mitigate the bioaccumulation of As, Cr, and Cu in plants. First, wastewater treatment plants (WWTPs) should implement tertiary treatment technologies, such as reverse osmosis, nanofiltration, activated carbon filtration, among others. Second, the absorption and translocation of these elements could be managed at the field level (Adiloğlu et al., 2020). Potential strategies that warrant further investigation include: (a) the selection of crop varieties with low heavy metal

accumulation capacity. For example, Zhou et al. (2022) reported that 49 maize varieties exhibited Cr concentrations below the Chinese MPL of 1 ppm, highlighting the importance of selecting varieties with low Cr accumulation potential for future treated wastewater studies; (b) the use of soil amendments such as biochar. Irfan et al. (2021) reported that biochar applied at a 4% rate reduced extractable Pb, Cd, and Cr concentrations by 79%, 61%, and 78%, respectively, likely due to immobilization of these metals in the soil, while simultaneously increasing soil organic matter and total nitrogen; (c) silicon (Si) application. Silicon can reduce heavy metal uptake and translocation through the stimulation of chelating agents, increased soil pH, formation of silicon complexes, and enhancement of enzymatic activity (Emamverdian et al., 2018); (d) the addition of amendments such as lime, phosphate fertilizers, and organic matter (Pais and Benton, 2000); and (e) the use of microorganisms capable of biosorption, bioaccumulation, biotransformation, biovolatilization, and biomineralization of heavy metals (Tang et al., 2024).

4 Conclusions

Irrigation of maize with treated wastewater produced similar effects on ear production, yield, quality, and plant nitrogen status as irrigation with potable water. Treated wastewater supplemented with *Agave lechuguilla* Torrey extract (saponins) at a rate of 0.9 mL m^{-2} promoted greater ear length, whereas inoculation with plant growth-promoting bacteria (*Pseudomonas fluorescens* and *Raoultella terrigena*) did not improve the evaluated variables.

In addition, treated wastewater irrigation resulted in greater accumulation of As, Cr, Cu, Fe, Mn, and Mo in maize kernels. Among these elements, As, Cr, and Cu exceeded the maximum permissible limits. The use of treated wastewater supplemented with *Agave lechuguilla* Torrey extract (saponins) may represent a viable alternative for agricultural irrigation. However, advanced purification technologies must be implemented in wastewater treatment plants to remove heavy metals from treated effluents. Furthermore, field-level strategies to control heavy metal bioaccumulation in crops should be combined with wastewater reuse practices before this resource can be considered a sustainable alternative for agricultural production.

Declarations

No funding was received for conducting this study. The authors have no relevant financial or non-financial interests to disclose.

Conflict of Interest.

The authors declare no competing interests.

Author Contribution

J.C.R.O. Conceptualization, Writing – original draft, Methodology, A.A.R. Methodology, P.E.D.F., G.M.R. Funding acquisition, M.J.R.M. Writing and Edition, J.A.A.J. Conceptualization, A.J.E.G. Writing – original draft and Statistical Analysis. All authors reviewed the manuscript.

Acknowledgement

Autoproduction of Foods Center, Integral Family Development of San Luis Potosí state and Training Center in Protected Agriculture, Agronomy and Veterinary Faculty of the University Autonomus of San Luis Potosí.

Data Availability.

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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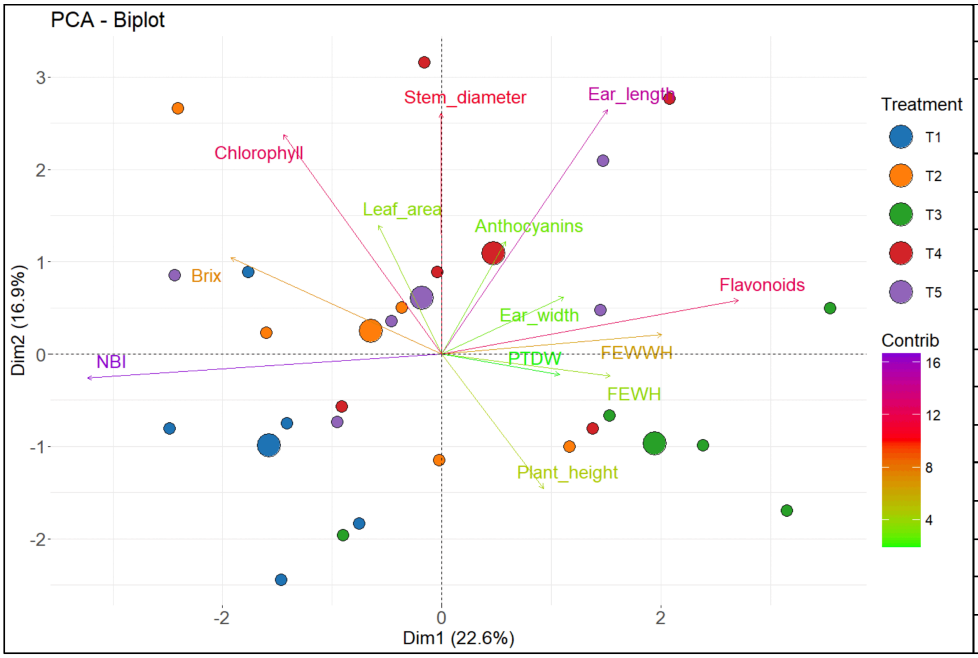
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Figures



Eigenvalue	2.94		2.19	
Variability (%)	22.64		16.89%	
Variable	Dim 1		Dim 2	
	%	F1	%	F2
NBI	28.85	-0.92	0.24	-0.07
Flavonoids	20.30	0.77	1.24	0.16
FEWWH	11.13	0.57	0.15	0.05
° Brix	10.22	-0.54	4.01	0.29
FEWH	6.51	0.43	0.21	-0.06
Ear length	6.32	0.43	25.91	0.75
Chlorophyll	5.76	-0.41	20.92	0.67
Ear width	3.42	0.31	1.40	0.17
PTDW	3.19	0.30	0.18	-0.06
Plant_height	2.39	0.26	7.85	-0.41
Anthocyanins	0.93	0.16	5.47	0.34
Leaf_area	0.91	-0.16	7.20	0.39
Stem_diameter	0.01	-0.002	25.14	0.74

Figure 1

Principal component analysis (PCA) of the different treated wastewater and potable water treatments. The colored arrows indicate the contribution of each variable. The table shows the contribution (%) of each variable to each dimension. FEWWH = Fresh ear weight with husks; FEWH = Fresh ear weight without husks; PTDW = Plant total dry weight