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INDUSTRIAL WASTEWATER, GROUNDWATER, AND BIOAUGMENTED BACTERIA EFFECT ON GROWTH AND HEAVY METAL ACCUMULATION IN RADISH

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SUMMARY

An examination of the effects of industrial wastewater and groundwater irrigation on the growth and heavy metal accumulation in radish (*Raphanus sativus* L.) was this study's focus. Conducted during 2023–2024 in Baiji, Salah Al-Din Governorate, Iraq, the study additionally assessed the three types of bacteria's role in reducing soil pollution and improving plant quality. Results indicated the average leaf height was 32.260 and 31.80 cm for industrial water and groundwater irrigation, respectively, enhancing to 34.7 and 38.0 cm with the addition of bacteria. Root length reached 11.70 and 8.70 cm with bacteria. The wet weight of leaves and roots was 32.600 and 49.00 g/plant, respectively, with industrial water, rising to 33.8 and 58.00 g/plant by adding bacteria. Nutrient concentrations (potassium, nitrogen, and phosphorus) in leaves and roots increased with industrial watering to 3.639%, 4.933%, 3.113%, 3.673%, 0.219%, and 0.634%, respectively. With bacteria, the potassium and nitrogen values rose to 3.700%, 5.233%, and 3.967%); however, the phosphorus values slightly lowered (0.201% and 0.631%). Heavy metal concentrations significantly decreased with bacteria, and minimum values recorded in industrial wastewater and groundwater irrigation were for lead (0.087 and 0.384 ppm), nickel (0.017 and 0.207 ppm), cadmium (0.079 and 0.093 ppm), and zinc (0.594 and 1.997 ppm).

Keywords: Radish (*R. sativus* L.), industrial wastewater, groundwater, bacterial bioaugmentation, growth traits, heavy metal accumulation

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Key findings: The addition of bacteria improved the growth traits and concentrations of primary nutrients in radish (*R. sativus* L.) irrigated with industrial wastewater and groundwater. The bacteria considerably helped in reducing the accumulation of heavy metals in leaves and roots. The results confirm the effectiveness of bacteria in improving plant quality and reducing soil pollution to enhance agricultural sustainability.

INTRODUCTION

Water is one of the most vital natural resources, essential for human life and other living organisms. However, the world is suffering from a severe decline in the quantity and quality of water because of climate change, increased population growth, and human activities, making us face more scarcity in the future (Santos *et al*., 2023). Recently, the treatment and reuse of wastewater in agriculture have become an urgent practice, especially in areas that suffer freshwater scarcity (Alwan and Saeed, 2024).

In general, wastewater contains various organic and inorganic nutrients essential for plant metabolism and growth. Moreover, wastewater containing toxic pollutants and heavy metals causes a serious problem due to toxicity and accumulation in the bodies of living organisms and their inability to biodegrade in the environment. Therefore, the long-term use of wastewater leads to soil pollution through the accumulation of micro-pollutants in the soil, such as heavy metals (Oubane *et al*., 2021).

The transmission of these pollutants through the food web to humans poses a real danger; hence the need to find appropriate solutions to treat these pollutants (Saroop and Tamchos, 2021). Bioremediation using microorganisms is an environmentally friendly and effective method for getting rid of pollutants in soil and water, which is low cost, compared with other treatment methods (Sarker *et al*., 2023). Several studies have reported that bacteria can easily fix heavy metals in the soil by enriching the cell surface, sedimentation, complex formation, oxidation, and reduction, which eventually reduce the toxicity of elements to crop plants (Han *et al*., 2020). The biological accumulation of heavy metals in arable soil represents a health risk due to their transmission to human bodies through the consumption of crops grown in contaminated fields (Ketema *et al*., 2023).

Radish (*Raphanus sativus* L.) is an annual crop belonging to the family Cruciferae (Brassicaceae), which has become one of the important root plants. Although its large taproot commonly appears as a root vegetable, the entire plant is edible, with its leaves sometimes used as a leafy vegetable. Radish consumption is mainly for foods due to its many nutrients, such as vitamin C, proteins, carbohydrates, and minerals (Gamba *et al*., 2021). Radish is a classified vegetable that excessively accumulates heavy elements. Therefore, the presented study aimed to determine the effects of industrial wastewater and groundwater irrigation on the growth and heavy metal accumulation in the radish (*R. sativus* L.). Additionally, the research sought to assess the role of three types of bacteria in reducing soil pollution and improving plant quality (Turek *et al*., 2019).

MATERIALS AND METHODS

Experimental procedure

The study experiment on radish (*R. sativus* L.) took place during 2023–2024. The 300 kg of sandy soil, taken from arable lands near the Baiji refinery in the north of Salah Al-Din Governorate, Iraq, was the soil type used in the said experiment. The soil sustained washing with distilled water several times to reduce impurities, then underwent air drying to be ready for planting (Taher and Saeed, 2023).

Soil and pot preparation

The soil sieving used a 4.50 mm diameter mesh to ensure the removal of coarse parts and ensure soil homogeneity. Afterward, the soil's equal distribution into 30 pots with dimensions (24 cm height × 30 cm diameter) transpired, with 10 kg of soil placed in each pot. The percentage of soil saturation with water reached estimation at 200 ml per kg of soil.

Seed planting

Radish (*R. sativus* L.) seeds, selected from the market of Tikrit City, Iraq, received sterilization with 0.5% potassium permanganate solution for 10 minutes before rinsing with distilled water three times to ensure their purity (Nayekova *et al*., 2024). The radish seeds' successful planting in pots began on October 25, 2023.

Irrigation treatments

Two types of water used for irrigation comprised a) industrial wastewater obtained from the Baiji refinery, Iraq, and b) groundwater from one of the wells of the crop area.

Addition of bacteria

The addition of three types of bacteria previously isolated and identified from the contaminated soil consisted of a) *Cytobacillus firmus* (IBMA-1), b) *Bacillus cereus* (IBMA-3), and c) *Bacillus zhangzhouensis* (IBMA-4). The bacteria's activation used a brain-heart infusion broth medium at a concentration of 0.5 nm, with the absorbance measured at a wavelength of 550 nm using a spectrophotometer. Adding 200 ml of bacterial solution per two liters of irrigation water continued to each pot (Hussein and Saeed, 2022).

Experimental treatments

The experiment included five main treatments. The first three are a) irrigation with industrial wastewater with the addition of three types of bacteria; b) irrigation with industrial wastewater with the addition of each type of bacteria separately, and c) irrigation with groundwater with the addition of three types of bacteria. The last two are 1) irrigation with groundwater with the addition of each type of bacteria separately and 2) irrigation with industrial wastewater and groundwater without the addition of bacteria (control).

Measurement of plant characteristics

In radish, the root length, as measured from the soil level to the tip of root, used a tape measure. Plant height's measurement started from the soil level to the top of the leaf (Fort and Freschet, 2020). Wet and dry weight of radish plants gained measuring after washing and drying. Samples' drying ensued in an oven at 75 °C for 72 h, before the dry plant underwent weighing using an electric scale (Kouki *et al.,* 2021).

Analysis of nutrients and heavy metals for plants

The potassium determination used a flame photometer after digesting the samples with sulfuric acid, where measuring the intensity of the radiation emitted by the potassium atoms proceeded. The determination of nitrogen applied the Berthelot reaction after digesting the samples with sulfuric acid with digestion aids, where the results received readings at the wavelength of 660 nm. The phosphorus, determined using Barton's reagent, depended on the reaction between phosphorus, vanadate, and molybdate, with the resulting compound estimated at the wavelength of 430 nm (Kashyap *et al*., 2016). The heavy elements' (heavy metals) extraction from the plant utilized nitric and perchloric acids. After preparation, the metal concentrations' estimation employed an atomic absorption spectrometer, with the results expressed in ppm (Taher and Saeed, 2022).

RESULTS AND DISCUSSION

Plant height and root length in radish

The results showed that in radish plants, the plant height bore considerable effects from the irrigation water types and the addition of bacteria (Table 1). The tallest plant and leaf

Table 1. Average leaf height as affected by irrigation water quality and bacteria.

Water type	Bacterial strains				IBMA-1+3+4	Means
	No bacteria	IBMA-1	IBMA-3	IBMA-4		
Well water	33.3b	28.7d	30.0cd	29.0d	38.0a	31.80a
Industrial water	32.7b	32.3bc	30.0cd	32.3bc	34.0b	32.26a
Means	33.00b	30.50b	30.00b	30.65b	36.00a	

Table 2. Average root length affected by irrigation water quality and bacteria.

Water type	Bacterial strains				IBMA-1+3+4	Means
	No bacteria	IBMA-1	IBMA-3	IBMA-4		
Well water	9.00e	10.00d	11.30ab	10.00d	8.70e	9.80a
Industrial water	10.30cd	9.30e	11.70a	9.00e	10.70bc	10.20a
Means	9.65b	9.65b	11.50a	9.50b	9.70b	

height (38.0 cm) appeared with groundwater irrigation and the addition of the three bacterial types together (IBMA-1, IBMA-3, and IBMA-4). The enhancement in plant height is ascribable to the decrease in heavy metal concentration in the leaves and the increase in nutrients (Tables 6 and 7). Past studies also revealed bacteria considerably contributed to reducing the accumulation of toxic heavy metals and improving the availability of nutrients to crop plants (Gkorezis *et al*., 2016). In contrast, the plant height decreased (28.7 cm) by irrigation with groundwater and the addition of one bacteria (IBMA-1), which can correlate with the increased heavy metal concentration and the decrease in nutrients (Table 7), with the same findings reported by Hermanto *et al*. (2021).

By using industrial water, a decrease was evident in the plant height as compared with groundwater irrigation. In the case of bacteria, the topmost plant height (34.0 cm) resulted from the application of three bacterial types collectively (Table 1). This may be due to the ability of bacteria to absorb heavy elements, mitigate the negative impact of wastewater, and cause an increase in plant biomass (Han *et al*., 2018). The results further revealed that in radish plants, the maximum root length (11.30 cm) came from irrigation with groundwater with the addition of IBMA-3 bacteria, while the root length decreased (8.70 cm) by using three types of bacteria together (Table 2). In industrial water, the longest root length (11.70 cm) was notable with the addition of IBMA-3 bacteria, while the shortest root length (9.0 cm) emerged with the addition of bacteria IBMA-4. The reason for the decrease in root length may be that one type of bacteria cannot absorb heavy metals significantly, as well as the negative interference in the mechanisms of interaction between bacteria and crop plants (Rasheed *et al.,* 2024a).

Wet and dry weight of radish plants

The results enunciated that the wet weight of leaves and roots in radish plants was higher in irrigation made with industrial water, reaching 35.333 and 63.667 g/plant, respectively, than in groundwater (33.767 and 52.333 g/plant, respectively) (Table 3). The reason for this may be with the positive role of bacteria in improving nutrient absorption and reducing the effect of heavy metals on plants, leading to an increase in plant biomass (Hermanto *et al*., 2021).

As for the radish plant's dry weight (Table 4), the irrigation made with industrial water led to the highest values of dry leaves and roots (4.667 and 2.327 g/plant, respectively), compared with the groundwater (4.033 and 2.567 g/plant, respectively) (Table 4). The difference in wet and dry weight values among the plants may be because the water mass in plant tissues increases disproportionately in the different plant tissues (Huang *et al*., 2019).

Table 3. Average wet weight of leaves and roots affected by irrigation water quality and bacteria.

Traits	Water type	Bacterial strains				IBMA-1+3+4	Means
		No bacteria	IBMA-1	IBMA-3	IBMA-4		
Shoots	Well water	32.233bc	26.400ef	28.333de	24.667f	33.767ab	29.080b
	Industrial water	35.333a	33.800ab	31.000bc	32.000bc	30.867cd	32.600a
	Means	33.783a	30.100b	29.667b	28.334b	32.317a	
Roots	Well water	52.333b	35.467e	37.433de	34.033e	40.267cd	39.907b
	Industrial water	63.667a	43.900c	47.267b	49.000b	41.167cd	49.000a
	Means	58.000a	39.684b	42.350b	41.517b	40.717b	

Table 4. Average dry weight of leaves and roots affected by irrigation water type and bacteria.

Traits	Water type	Bacterial strains				IBMA-1+3+4	Means
		No bacteria	IBMA-1	IBMA-3	IBMA-4		
Shoots	Well water	2.100a	1.700a	1.833a	1.733a	2.133a	1.859a
	Industrial water	2.327a	2.220a	2.010a	2.033a	1.953a	2.109a
	Means	2.214a	1.960a	1.922a	1.883a	2.043a	
Roots	Well water	4.033ab	2.133de	2.267de	2.067e	2.567de	2.613b
	Industrial water	4.667a	3.100c	3.733b	3.767b	2.667d	3.587a
	Means	4.350a	2.617b	3.000b	2.917b	2.617b	

Nutrients' concentration

The findings revealed that the adding of bacteria led to an increase in potassium and nitrogen concentrations in the radish plant leaves (Table 5). The radish plants irrigated with groundwater resulted in potassium and nitrogen concentrations increasing from 2.533% and 2.333% to 2.800% and 2.700%, respectively, while the phosphorus content decreased from 0.3853% to 0.1117% by adding three types of bacteria.

By using industrial water, the results exhibited an escalation in potassium and nitrogen concentrations (3.700% and 3.300%), with a slight decrease in phosphorus compared with groundwater. The potassium and nitrogen concentrations in the roots of radish plants irrigated with groundwater increased by adding bacteria from 3.467% and 2.7330% to 3.700% and 3.0000%, while phosphorus considerably decreased from 0.6817% to 0.4510%. As for the roots of radish plants irrigated with industrial water, the concentration of potassium and nitrogen rose from 4.533% and 3.5670% to 5.233% and 3.9670%. Meanwhile, a decrease was evident in phosphorus concentration from 0.8563% to 0.5240%. As shown in Table 6, this may be due to bacteria contributing to fixing nitrogen in the soil and consuming some part of the phosphorus used in different processes initiated in crop plants (Benjelloun *et al*., 2021).

Heavy elements

The obtained data indicated a remarkable reduction in the heavy metal concentration in radish plant leaves with the application of bacterial treatments (Table 7). For instance, the concentration of lead decreased notably from 0.1625 ppm (in untreated plants) to 0.0333 ppm (with combined application of three bacterial strains). Similar trends were prominent for nickel, cadmium, and zinc; their concentrations in the radish plant leaves diminished by treatment with bacteria compared with plants irrigated with either groundwater or industrial water.

Similarly, the study results further revealed a reduction in heavy metal concentration in radish plant roots upon bacterial treatment. The lowest level manifested with the combined application of three bacterial strains (Table 8). For example, the cadmium level in radish plant roots declined from 0.2270 to 0.1002 ppm using

Table 5. Average concentration of potassium, nitrogen, and phosphorus in leaves as affected by irrigation water quality and bacteria.

Traits	Water type	Bacterial strains				IBMA-1+3+4	Means
		No bacteria	IBMA-1	IBMA-3	IBMA-4		
K %	Well water	2.533b	1.833c	1.800c	1.633c	2.800b	2.1198b
	Industrial water	3.633a	3.700a	3.633a	3.633a	3.600a	3.6398a
	Means	3.083a	2.7665a	2.7165a	2.633a	3.200a	
N %	Well water	2.333d	2.600cd	2.600cd	2.533d	2.700bcd	2.5532b
	Industrial water	3.000ab	3.300a	3.100ab	2.933abc	3.233a	3.1132a
	Means	2.667a	2.950a	2.850a	2.733a	2.967a	
P %	Well water	0.3853a	0.1317cd	0.1213cd	0.1190d	0.1117d	0.1738b
	Industrial water	0.3513a	0.2010b	0.1977b	0.1947b	0.1543c	0.2198a
	Means	0.3683a	0.16635b	0.1595b	0.15685b	0.1330b	

Table 6. Average concentrations of potassium, nitrogen, and phosphorus in plant roots as affected by irrigation water quality and bacteria.

Traits	Water type	Bacterial strains				IBMA-1+3+4	Means
		No bacteria	IBMA-1	IBMA-3	IBMA-4		
K %	Well water	3.467c	3.367c	3.300c	3.467c	3.700c	3.460b
	Industrial water	4.533b	4.900ab	5.233a	4.900ab	5.100a	4.933a
	Means	4.000a	4.134a	4.267a	4.184a	4.400a	
N %	Well water	2.7330bc	2.5670bc	2.8670bc	2.4000c	3.0000ab	2.7134b
	Industrial water	3.5670a	3.4000ab	3.7330a	3.9670a	3.7000a	3.6734a
	Means	3.1500a	2.9835a	3.3000a	3.1835a	3.350a	
P %	Well water	0.6817b	0.5580d	0.4750ef	0.4956ef	0.4510f	0.5323b
	Industrial water	0.8563a	0.6310bc	0.5820cd	0.5810cd	0.5240de	0.6349a
	Means	0.7690a	0.5945b	0.5285c	0.5383c	0.4875c	

Table 7. Average of heavy elements Pb, Ni, Cd, and Zn in plant leaves affected by irrigation water quality and bacteria.

Traits	Water type	Bacterial strains				IBMA-1+3+4	Means
		No bacteria	IBMA-1	IBMA-3	IBMA-4		
Pb	Well water	0.1625b	0.0487d	0.0458d	0.0433d	0.0333d	0.0667b
	Industrial water	0.3850a	0.1046c	0.0982c	0.0975c	0.0791cd	0.1529a
	Means	0.2738a	0.0767b	0.0720b	0.0704b	0.0562b	
Ni	Well water	0.0201b	0.0153b	0.0147b	0.0126b	0.0108b	0.0147b
	Industrial water	0.0838a	0.0205b	0.0206b	0.0204b	0.0172b	0.0325a
	Means	0.0519a	0.0179b	0.0177b	0.0165b	0.0140b	
Cd	Well water	0.1625b	0.0487d	0.0458d	0.0433d	0.0333d	0.0667b
	Industrial water	0.3850a	0.1046c	0.0982c	0.0970cd	0.0796c	0.1529a
	Means	0.2738a	0.0767b	0.0720b	0.0701b	0.0564b	
Zn	Well water	0.9091b	0.5501cd	0.5107cd	0.4921cd	0.3742d	0.5672b
	Industrial water	1.2314a	0.8233b	0.7781bc	0.8060b	0.5941c	0.8466a
	Means	1.0703a	0.6867b	0.6444b	0.6491b	0.4842c	

groundwater as the irrigation source. These reductions in heavy metals in different plant parts can refer to the ability of bacteria to immobilize heavy metals within the soil and transform them into non-bioavailable forms, thereby limiting their uptake and accumulation in plant tissues. This mechanism was greatly analogous to findings reported by Gonzalez and Ghneim-Herrera (2021), with further support from Rasheed *et al*. (2024b). They also highlighted the considerable positive role of specific bacterial strains in enhancing soil remediation processes, particularly through metal stabilization and reduction of phyto-availability, contributing to reduced metal translocation in crop plants.

Table 8. Average of heavy elements Pb, Ni, Cd, and Zn in plant roots affected by irrigation water quality.

Traits	Water type	Bacterial strains				IBMA-1+3+4	Means
		No bacteria	IBMA-1	IBMA-3	IBMA-4		
Pb	Well water	0.6095b	0.1018c	0.1025c	0.0913c	0.3236b	0.2457b
	Industrial water	1.4921a	0.5093b	0.4456b	0.3849b	0.5006b	0.6665a
	Means	1.0508a	0.3056b	0.2741b	0.2381b	0.4121b	
Ni	Well water	0.2130b	0.1157c	0.1053c	0.1073c	0.1213c	0.1325b
	Industrial water	0.4656a	0.2397b	0.2327b	0.2070b	0.4240a	0.3138a
	Means	0.3393a	0.1777c	0.1690c	0.1572c	0.2727b	
Cd	Well water	0.2270d	0.1107e	0.1037e	0.1439	0.1002e	0.1371b
	Industrial water	0.7082a	0.3097c	0.2389d	0.4074b	0.0935f	0.3515a
	Means	0.4676a	0.2102c	0.1713c	0.2757b	0.0969d	
Zn	Well water	2.074b	1.343c	1.248c	1.239c	1.216c	1.424
	Industrial water	3.800a	2.025b	2.105b	2.114b	1.997b	2.408
	Means	2.937a	1.684b	1.677b	1.677b	1.607b	

CONCLUSIONS

The study demonstrated the importance of using bacteria to improve radish plant growth grown in polluted soils, as they significantly contributed to reducing the accumulation of heavy metals and improving the absorption of nutrients by the plants. These results could provide sustainable solutions to address the environmental threats associated with irrigation with industrial wastewater.

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