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Using Bioremediation to Reduce Groundwater Salinity

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Abstract

A study was conducted to evaluate the effectiveness of microorganisms in reducing groundwater salinity and to identify the most effective organisms. A laboratory experiment was performed using a completely randomised block design with a factorial arrangement, with four treatments and three replicates, yielding 12 experimental units for the biological treatment of groundwater. This experiment included the sequential addition of microorganisms (control, *Providencia rettgeri, Bacillus amyloliquefaciens*, and *Rhizoctonia solani*) to groundwater with a salinity of 13108 µS/cm, with continuous monitoring of changes in EC, pH, and other water chemical properties. *Providencia rettgeri* achieved the highest temperature on June 26th, reaching 29.0 °C, whereas the control treatment recorded a lower temperature on the same date. Meanwhile, *Bacillus amyloliquefaciens* outperformed the control, achieving the highest nitrogen content in the treated water at 1499 mg/L, compared with the control at 1372 mg/L. *Rhizoctonia solani* excelled by recording the lowest electrical conductivity and pH for the treated water on 6/25, at 11607 ds/cm and 6.73, respectively, compared to the control treatment, which recorded the opposite in both characteristics.

Keywords: *Bacillus amyloliquefaciens,* bioremediation, groundwater, *Providencia rettgeri*

Introduction

Bioremediation technologies play a crucial role in addressing environmental challenges posed by climate change, overuse of saltwater, and rising groundwater salinity resulting from unsustainable agricultural expansion. These strategies harness the power of microorganisms or plants, combined with biotechnology, to transform saline environments into healthier, more usable aquatic systems (El-Sayed et al., 2025; Wang & Zhang, 2025).

On the other hand, the use of specialized salt-tolerant microorganisms, such as *Haloarchaea*, is a promising option for treating brackish water and industrial wastewater. These microorganisms possess a unique ability to adapt to extreme salinity conditions, making them ideal natural candidates for bioremediation applications in such environments (Fernández & Ventosa, 2025; Patel & Singh, 2024). Another recent trend is the use of microbial desalination cells (MDCs), which use microorganisms to generate an electrical current, thereby facilitating the desalination of groundwater. These cells offer a sustainable solution that combines biological treatment with clean energy (Zhao et al., 2025).

Groundwater is one of the most critical water resources on earth. It is considered a strategic reserve in many regions of Iraq and the world, accounting for approximately 30% of available water resources. It can play a vital role in supporting environmental and human life. The importance of groundwater is highlighted by its role in stabilizing surface water levels by feeding rivers and lakes. It also serves as an alternative solution to meet community needs amid rapid population growth, scarce water resources, and their mismanagement (Hossain et al., 2022).

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The problem of groundwater salinity is one of the obstacles to agricultural development, primarily due to scarce water supplies, high temperatures, evaporation, inefficient irrigation systems, and excessive irrigation, which can draw large amounts of accumulated salt into the depths. Furthermore, soil and rocks contain salts that can decompose and disintegrate due to natural processes and enter groundwater, among other reasons (Ullah et al., 2019), Recent research has indicated the potential for using fungal strains in integrated biological control (IBC) programs when combined with effective microorganisms such as *Bacillus velezensis*, which has shown effectiveness in inhibiting the growth of *R. solani*, particularly group AG-4, one of the most dangerous pathogens of potatoes and tomatoes (Lee et al., 2023).

Bacillus amyloliquefaciens is a beneficial bacterium that plays a key role in organic agriculture and the environment. This bacterium is characterized by its ability to produce a wide range of enzymes, organic acids, and antimicrobial compounds, making it an effective agent in controlling fungi and plant-pathogenic bacteria (Al-Hadawi, 2018). It also stimulates plant growth by improving nutrient availability and enhancing plant resistance to environmental diseases (Al-Obaidi, 2019).

In the context of efforts to reduce reliance on chemical fertilizers and pesticides, non-pathogenic beneficial bacteria have become among the most prominent microorganisms receiving widespread attention in sustainable agriculture. Among these bacteria, *Providencia rettgeri* stands out. Some strains of this bacterium, isolated from soil and the rhizosphere, were found to lack known virulence factors, making them strong candidates for bio-agricultural applications (Chaudhary et al., 2022). Bacteria-based water remediation is well established at concept and lab scale, but there are gaps around field performance, ecosystem safety, and its integration with technology. Our study investigated the role of various microorganisms in modulating the chemical balance of saline groundwater, aiming to identify superior isolates for biological water treatment.

Materials and Methods

Preparation of ***Providencia rettgeri*** Bacteria

A group of salt-tolerant *Providencia rettgeri* bacteria was isolated from the rhizosphere of the tartar plant at the College of Agriculture, University of Kufa. *Providencia rettgeri* is characterized by its high salinity tolerance. Media specific to the bacteria (nutrient agar) was prepared according to the concentrations recommended on the package. The media were placed in an autoclave for sterilization. Samples were taken, and seven dilutions were prepared in dilution tubes using distilled water. Samples were then taken from these dilutions and placed in their respective media. They were then incubated for 48 hr, after which they were identified (Figure 1).

Preparation of ***Bacillus amyloliquefaciens*** Culture

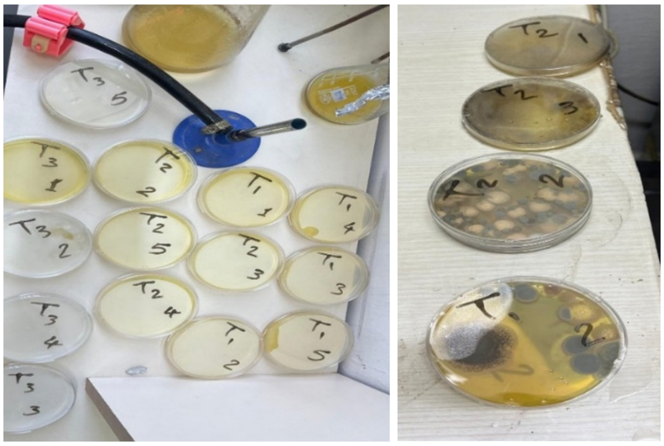
Samples were collected from saline soil at the College of Agriculture, University of Kufa. Seven dilutions were made, from which samples were taken and placed in their respective media, which had been prepared and sterilized in advance. The samples were incubated for 48 hr, after which they were identified.

Preparation of ***Rhizoctonia solani*** Culture

Rhizoctonia solani was used and activated with millet seeds, which were thoroughly washed with water to remove dirt and impurities. The seeds were soaked in a glass beaker for 6 hr, after which the water was removed, and the seeds were left on a piece of gauze for an hour to drain excess water. The seeds were placed in 250 ml glass beakers, 50 g per beaker. The seeds were sterilized in an autoclave at 121 °C and 15 psi for 1 hr. The sterilization process was repeated the next day at the same temperature, pressure, and time. They were left to cool and then refrigerated until use (Dewan, 1989).

Figure 1

Preparations of Providencia rettgeri Bacterial Culture for the Experiment



Procedures

On June 17, 2025, the experimental setup was established using four glass basins, each partitioned into three equal sections. The basins were pre-treated and rinsed to ensure the removal of all surface contaminants. To mitigate high baseline salinity, the source water (initial electrical conductivity, EC 13,108 dS/cm) was diluted 1:3 with deionized water prior to tank allocation.

Each basin was filled with 40 L of the diluted, filtered water. To monitor the chemical and physical stability of the system, key parameters—including pH, EC, temperature, and macronutrient concentrations (N, P, and K)—were recorded at frequent intervals. Measurements were taken on June 17, 19, 22, 23, 24, and 25 to capture the precise fluctuations in water chemistry both before and after the experimental treatments.

Preparation of Bacterial Inocula

Six 250 ml glass bottles were prepared and filled with 200 ml of distilled water. Nutrient Broth medium was added at a concentration of 2.6 ml (per manufacturer specifications). The medium was sterilized via autoclaving at 121 °C for 60 min. Under aseptic conditions, three bottles were inoculated with 10 ml of a pre-cultured *Providencia rettgeri* suspension. The remaining three bottles were inoculated with 10 ml of a pre-cultured Bacillus amyloliquefaciens suspension. All cultures were incubated at 37 °C for 48 hr to reach the desired bacterial density.

Preparation of Fungal Culture (PDA Medium)

Potato dextrose agar (PDA) was prepared by dissolving 8.2 g of PDA in 200 ml of distilled water within three glass jars. The medium was sterilized in an autoclave for 60 min and then poured into sterile petri dishes under a laminar-flow hood. After solidification, the plates were inoculated with *Rhizoctonia solani* and incubated at 27 °C for 72 hr.

Physicochemical Analysis of Water

Water quality parameters were monitored throughout the experimental period. Temperature was recorded with a standard thermometer, while electrical conductivity (EC) and pH were measured with digital meters calibrated according to the World Health Organization (2017) and International Organization for Standardization (2008) standards, respectively.

Determination of Total Nitrogen (TN)

Total Nitrogen was quantified using a modified Kjeldahl method (American Public Health

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Association, American Water Works Association, & Water Environment Federation, 2017). This procedure ensures that all nitrogenous forms are converted to ammonia for titration. Digestion: Organic nitrogen was converted to ammonium sulfate using sulfuric acid (H_2SO_4) and a catalyst. Reduction: Following digestion and cooling, a Devarda's alloy mixture was added as a reducing agent to convert nitrates (NO_3) and nitrites (NO_2) into ammonia/ammonium, a step essential for capturing total nitrogen. Distillation: The sample was alkalized with sodium hydroxide (NaOH), and the liberated ammonia was steam-distilled into a boric acid indicator solution. Titration: The solution was titrated against standard hydrochloric acid (HCl). TN concentration was calculated based on the acid titer and expressed in parts per million (ppm).

Determination of Phosphorus and Potassium

Total dissolved phosphorus (P) was assessed colorimetrically using a spectrophotometer according to the protocols of the American Public Health Association, American Water Works Association, & Water Environment Federation (2017). Total dissolved potassium (K) was determined using a flame photometer (American Public Health Association, American Water Works Association, & Water Environment Federation, 2017), utilizing specific emission wavelengths for quantification.

Statistical Analysis of Experimental Data

A completely randomized design was used, and the data were analyzed using Genstat for ANOVA. The means of the treatments were compared using the LSD test (Al-Rawi & Allah, 1980).

Results and Discussion

Effects of Biological Treatments on Water Temperature

The analysis of variance presented in Table 1 revealed significant temporal fluctuations in water temperature, primarily driven by ambient meteorological conditions during the study period. The minimum average temperature was recorded on June 22 (20.68 °C), while the maximum occurred at the onset of the experiment on June 17 (29.85 °C). Statistical analysis showed no significant differences among treatments, confirming that all experimental units were subjected to uniform thermal environmental loads.

Despite the lack of statistical significance between groups, the *Providencia rettgeri* treatment consistently showed the lowest absolute temperatures. This cooling effect can be attributed to the reduction of the organic load and total suspended solids (TSS) within the aqueous medium. Biological degradation of organic matter improves water clarity by reducing turbidity.

Lower turbidity reduces the absorption of solar radiant heat, as fewer suspended particles are available to act as "heat sinks." Furthermore, the metabolic consumption of complex organic compounds by *P. rettgeri* facilitates the precipitation of biomass. This reduction in dissolved organic matter diminishes the water column's heat retention capacity, allowing more efficient heat dissipation to the surroundings via surface exchange. Consequently, the relative decrease in temperature serves as an indirect indicator of enhanced water quality and successful pollutant mitigation.

The Effect of Biological Treatments on Water Electrical Conductivity (EC)

The application of biological treatments significantly reduced dissolved salt accumulation in the water compared to the control (Table 2). Treatment of *Rhizoctonia solani* demonstrated the highest efficacy in reducing EC, indicating a robust capacity to mitigate ionic effects. Interestingly, this treatment also correlated with the observed reduction in water temperature. This suggests that biological interventions can reorganize the ionic structure of the aqueous medium, which indirectly influences thermal properties by enhancing complex biological

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interactions (Mahadevaiah et al., 2023).

Biologically treated irrigation water has been shown to decrease soluble salt concentrations, a critical factor for drip or limited-leaching irrigation systems where salt buildup is common (Rengasamy, 2010). The observed salinity reduction and temperature stabilization reflect the dynamic interaction between the microorganisms and the water's chemical constituents.

While the treatments involving *Providencia rettgeri* and *Bacillus amyloliquefaciens* were effective, an upward trend in EC was noted between June 22 and June 25 (Table 2). This aligns with the gradual increase in ambient temperatures during that period. This phenomenon is a direct result of increased evaporation rates, which concentrate dissolved ions in the remaining water volume—a relationship well documented in previous studies (Rengasamy, 2010).

The Effect of Biological Treatments on Water pH

The biological treatments significantly influenced the pH of the treated water, as detailed in Table 3. The primary objective was to reduce pH to a slightly acidic or neutral range to optimize nutrient availability. Treatment with *Providencia rettgeri* was the most effective, reducing the pH from 7.60 to 6.53. This reduction is a direct consequence of high microbial activity during the decomposition of organic matter, or biolysis. As bacteria metabolize organic substrates, they produce various organic acids, including volatile

Table 1

The Effect of Biological Treatments on Water Temperature (°C)

Treatment	Temperature (°C)						Average temperature (°C)	Temperature reduction (°C)
	17-June	19-June	22-June	23-June	24-June	25-June		
Control	30.20	28.80	21.00	24.00	27.90	28.60	26.76	-0.6
Providencia rettgeri	30.00	28.70	21.20	26.80	25.80	29.00	26.91	-1.0
Bacillus amyloliquefaciens	29.70	28.60	20.50	25.90	27.50	28.80	26.82	-0.9
Rhizoctonia solani	29.50	28.40	20.00	24.80	27.30	28.70	26.45	-0.8
Average dates	29.85	28.61	20.68	25.38	27.11	28.78		
LSD 0.05	Treatments		Data		Interference			
	0.755		0.925		1.85			

Table 2

The Effect of Biological Treatments on the Water Electrical Conductivity (dS/cm)

Treatment	Water EC (dS/m)						Average (dS/m)
	17-June	19-June	22-June	23-June	24-June	25-June	
Control	13,228	13,889	14,395	14,775	17,895	18,908	15,515
Providencia rettgeri	13,168	12,088	12,754	13,769	13,288	14,566	13,272
Bacillus amyloliquefaciens	12,988	12,388	12,988	11,908	12,328	13,708	12,718
Rhizoctonia solani	11,961	12,928	10,620	11,067	11,127	11,607	11,552
Average dates	12,837	12,823	12,689	12,880	13,660	14,697	
LSD 0.05	Treatments		Data		Interference		
	744.1		911.3		1822.7		

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fatty acids and other acidic intermediates. The dissociation of these compounds increases the concentration of hydrogen ions (H^+) in the medium, effectively lowering the pH.

Furthermore, microbial respiration significantly increases dissolved carbon dioxide (CO_2) levels in the water. When CO_2 dissolves, it reacts with water to form carbonic acid (H_2CO_3), which further acidifies the aqueous environment. The treatment also affected the water's alkalinity (buffering capacity). As bacteria consume natural bases during decomposition and potentially initiate partial nitrification, the water's acid-regulating capacity is diminished, leading to a more pronounced decrease in pH. While transient fluctuations may occur if certain bacterial enzymes neutralize acidic ions, the long-term metabolic output typically drives the system toward an acidic state as alkalinity declines.

In contrast, treatment with *Bacillus amyloliquefaciens* showed a more moderate effect on pH. This suggests a more balanced metabolic profile for this species in this specific medium, maintaining a relatively stable pH compared to the aggressive acidification observed with *P. rettgeri*. These results align with those of Xu et al. (2021), confirming that microbial management can effectively modify water chemistry for agricultural use without significantly altering pH.

Effect of Biological Treatments on Total Nitrogen (TN) Content

The analysis of water nitrogen levels (Table 4) revealed a gradual and significant increase in TN concentrations across the biological treatments over the experimental period. This accumulation is primarily attributed to the metabolic activity of the introduced microorganisms, which facilitates the mineralization of organic matter or the fixation of atmospheric nitrogen into soluble aqueous forms.

The bacterial treatments, specifically *Providencia rettgeri* and *Bacillus amyloliquefaciens*, demonstrated a pronounced capacity to increase total water nitrogen. In contrast, the presence of the fungus *Rhizoctonia solani* led to a localized reduction in available nitrogen. This decrease is likely due to nitrogen immobilization, where the fungus assimilates soluble nitrogen into its developing biomass to support hyphal growth.

Furthermore, the saline stress imposed by the experimental conditions may have modulated nitrogen balance. High salinity can suppress the activity of specific nitrogen-fixing microbes while potentially accelerating nitrogen loss via nitrification and denitrification. The varying responses observed across treatments confirm that the selection of specific microbial strains is a critical determinant of the aquatic nitrogen balance, a finding consistent with the foundational work of Reddy and Patrick (1984).

The high nitrogen levels observed across all treatments, including the control, may further be explained by the high osmotic pressure of the saline environment. Such stress can lead to the lysis or partial decomposition of microbial cells, subsequently releasing their internal organic nitrogen into the water column. Additionally, the controlled laboratory setting likely minimized natural nitrogen losses typically associated with field conditions, such as leaching, deep seepage, and ammonia volatilization (Yan et al., 2015).

Effect of Biological Treatments on Water Phosphorus Content

The concentration of dissolved phosphorus in the biologically treated water exhibited a significant upward trend across all treatments. By the third day of the experiment (June 22), phosphorus levels reached the maximum detection limit (1999 mg/L), a threshold maintained through the final measurement on June 25 (Table 5).

Among the microbial agents, *Providencia rettgeri* initiated the earliest release of phosphorus into the water column. However, the *Bacillus* treatment ultimately achieved a greater overall increase. This phenomenon is likely driven by the secretion of phosphatase enzymes and organic acids by these bacteria. These biochemical agents act on insoluble phosphate

Table 3

Effect of Biological Treatments on the pH of Treated Water

Treatment	Water pH						Average pH	pH reduction
	17-June	19-June	22-June	23-June	24-June	25-June		
Control	7.07	7.07	8.23	6.23	6.50	5.17	6.71	-1.90
Providencia rettgeri	7.60	7.40	7.03	6.67	7.20	6.53	7.07	-1.07
Bacillus amyloliquefaciens	7.43	6.80	6.73	6.53	7.33	6.60	6.91	-0.51
Rhizoctonia solani	7.30	6.77	6.57	6.47	7.67	6.73	6.92	-0.38
Average dates	7.35	7.01	7.14	6.48	7.18	6.26		
LSD 0.05	Treatments		Data		Interference			
	0.225		0.275		0.550			

Table 4

Effect of Biological Treatments on the Water Total N Content (mg/L)

Treatment	Total nitrogen content (mg/L)						N average (mg/L)
	17-June	19-June	22-June	23-June	24-June	25-June	
Control	516	829	1,194	1,557	1,389	1,372	1,143
Providencia rettgeri	711	770	1,015	1,268	1,343	1,408	1,086
Bacillus amyloliquefaciens	504	809	1,347	1,435	1,271	1,499	1,144
Rhizoctonia solani	459	792	1,065	1,173	1,292	1,318	1,017
Average dates	547	800	1,155	1,358	1,324	1,399	
LSD 0.05	Treatments		Data		Interference		
	96.9		118.7		237.3^{ns}		

Table 5

Effect of Biological Treatments on the Water Phosphorus Content (mg/L)

Treatment	Water phosphorus content (mg/L)					
	17-June	19-June	22-June	23-June	24-June	25-June
Control	1,239	1,962	1,999	1,999	1,999	1,999
Providencia rettgeri	1,690	1,835	1,999	1,999	1,999	1,999
Bacillus amyloliquefaciens	1,211	1,913	1,999	1,999	1,999	1,999
Rhizoctonia solani	1,098	1,877	1,999	1,999	1,999	1,999
Average dates	1,309	1,896	1,999	1,999	1,999	1,999
LSD 0.05	Treatments		Data		Interference	
	29.65		36.32		72.63	

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complexes, converting them into plant-available, soluble forms. These results align with the findings of Rodríguez and Fraga (1999) and Khan et al. (2025), who documented the efficacy of Phosphorus-Solubilizing Microorganisms (PSMs) in enhancing nutrient availability in aquatic and soil-water systems.

The observed increase in phosphorus was synchronized with the rising nitrogen levels and the previously discussed pH modifications. This correlation confirms that the biological treatments effectively transformed the chemical environment, enhancing the solubility of essential macronutrients. The ability of these specific bacterial strains to maintain such high dissolved phosphorus levels underscores their potential as biofertilizers in water management strategies (Al-budairy & Al-Taweel, 2025).

Effect of Biological Treatments on Water Potassium (K) Content

The results presented in Table 6 indicate that incorporating biological treatments into saline water significantly increased dissolved potassium concentration. This accumulation is attributed to the specialized metabolic activities of the introduced microorganisms, which facilitate the release of potassium from suspended mineral particles and organic fractions through biosolubilization.

The bacteria likely secreted a range of organic acids that lowered the localized pH at the mineral-water interface, combined with complex mechanisms such as chelation and ion exchange. These processes effectively displace potassium ions from the surfaces of suspended solids into the aqueous phase. Furthermore, the observed pH changes significantly enhanced the thermodynamic solubility of potassium, a finding that aligns with recent studies by Wang et al. (2025) and Al-Fatlawi and Mahmood (2025).

The ability of biological treatments to increase available potassium is particularly significant for saline water management. Increasing the Potassium-to-Sodium (K^+/Na^+) ratio in irrigation water is a known strategy to mitigate the deleterious effects of salinity on soil structure and plant physiology (Silva & Torres, 2025). Therefore, these results suggest that microbial pre-treatment of saline water can transform it into a more balanced nutrient solution, potentially reducing the osmotic and ionic stress on crops in later growth stages.

Conclusions

Based on the findings of this laboratory experiment, we conclude that biological treatments are effective at modulating the physicochemical and nutrient profiles of saline water, underscoring the strategic value of microbial pretreatment. Specifically, water treated

Table 6

Effect of Biological Treatments on the Treated Water Potassium Content

Treatment	K content (mg/L)					
	17-June	19-June	22-June	23-June	24-June	25-June
Control	1,243	1,969	1,997	1,997	1,997	1,997
Providencia rettgeri	1,695	1,834	1,999	1,999	1,999	1,999
Bacillus amyloliquefaciens	1,213	1,920	1,999	1,999	1,999	1,999
Rhizoctonia solani	1,099	1,884	1,999	1,999	1,999	1,999
Average dates	1,312	1,901	1,998	1,998	1,998	1,998
LSD 0.05	Treatments		Data		Interference	
	28.96		35.47		70.94	

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with the salt-tolerant bacterium *Providencia rettgeri* showed the greatest temperature reduction, likely due to reduced organic load and medium turbidity. Given its documented thermal stability, *P. rettgeri* represents a good candidate for bioremediation in high-temperature environments. Bioremediation using the fungus *Rhizoctonia solani* demonstrated distinct superiority in modifying chemical parameters, with the lowest final salinity (electrical conductivity) and the highest pH values recorded at the end of the experimental period. The ability of *R. solani* to thrive under saline conditions and to simultaneously increase water pH suggests its potential to improve both water and soil quality in salt-affected regions. Further studies should be conducted to expand the uses of *P. rettgeri* and *R. solani* to transform saline water into a more viable resource for sustainable agricultural irrigation.

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