Isolation and Identification of *Azotobacter chroococcum* Strain and Measuring Microbial Respiration in Saline Soil

Azza Massoud[1]

ABSTRACT

Free-living nitrogen-fixing *Azotobacter* bacteria are beneficial microorganisms that produce phytohormones, contributing to soil fertility and plant growth. It is well known that soil salinity causes significant disturbances in microbial ecosystems, affecting the biological functions of beneficial bacteria. Saline conditions, particularly high concentrations of sodium chloride (NaCl), can inhibit the growth and metabolic activity of these bacteria. This study investigates the viability and activity of *Azotobacter* in saline soils. It evaluates soil respiration—measured by carbon dioxide (CO_2) emission—as a key indicator of microbial activity under high soil salinity. By isolating and characterizing *Azotobacter* strains from El-Moghraa soil, adding them to highly saline soils, and measuring their microbial activity, we identified strains that can withstand harsh soil salinity conditions. The results demonstrated the biological activity of *Azotobacter* bacteria in the soil, highlighting their potential for use as biofertilizers in saline agricultural systems.

This research serves as a comprehensive evaluation of the role of a *Azotobacter chroococcum* strain, whose identity was confirmed through 16S rRNA gene sequencing, in improving the health of saline soil. The results clearly show that introducing this bacterium increases soil respiration even under high salinity conditions (EC = 14 dS/m), confirming its significant salt tolerance. Importantly, the addition of organic matter in the form of vermicompost significantly enhanced this effect, indicating a synergistic role. Vermicompost provides a source of carbon and nutrients, in addition to its role in mitigating the osmotic and ionic stress caused by salinity, thereby creating a protected microenvironment suitable for the microbes to flourish. This study presents a strong, evidence-based strategy for the sustainable management of soil in salinity-affected regions, offering a promising and eco-friendly alternative to traditional chemical inputs in agriculture.

Keywords: *Azotobacter*, saline soil, soil respiration, sodium chloride (NaCl), microbial activity, carbon dioxide (CO_2) emission, biofertilizers.

INTRODUCTION

Azotobacter and its role:

Azotobacter is considered one of the beneficial microorganisms. It is a Gram-negative, aerobic, diazotrophic bacterium that plays a vital role in the nitrogen cycle within the soil, thereby enhancing plant growth due to its ability to fix atmospheric nitrogen independently. In addition, it produces plant growth-promoting hormones such as cytokinins, gibberellins, and indole-3-acetic acid (Ahmad *et al.*, 2005).

Azotobacter is a genus of free-living, nitrogen-fixing bacteria widely recognized for its ecological and agricultural significance. These bacteria contribute to soil fertility by fixing atmospheric nitrogen and producing growth-promoting substances such as phytohormones and siderophores. Among the species, *Azotobacter chroococcum* is one of the most studied due to its robust nitrogen-fixation ability and adaptability to various soil environments. Accurate identification of *Azotobacter* species is essential for understanding their ecological roles and optimizing their use in biofertilizer formulations (Bhattacharyya & Jha, 2012; Wani *et al.*, 2013 and Romero-Perdomo *et al.*, 2017).

Traditional morphological and biochemical methods, while useful, often lack the resolution needed for precise taxonomic classification (Bhattacharyya & Jha, 2012; Wani *et al.*, 2013 and Romero-Perdomo *et al.*, 2017). Therefore, molecular techniques such as 16S rRNA gene sequencing have become indispensable tools for the reliable identification and phylogenetic analysis of bacterial isolates (Lane, 1991 and Sambrook & Russell, 2001). This technique targets a highly conserved gene present in all bacteria, allowing for accurate comparison and identification of species (Lane, 1991).

This study aimed to isolate *Azotobacter* strains and characterize them at the molecular level using 16S rRNA sequencing to confirm their taxonomic identity and assess their potential for agricultural applications. Moreover, *Azotobacter* improves soil structure and fertility through the production of exopolysaccharides, making it a promising candidate for sustainable agriculture as a biofertilizer and soil conditioner (Chennappa *et al.*, 2017 and Soniari & Atmaja, 2019).

The challenge of soil salinity and the role of *Azotobacter*:

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[1] Department of Soil & Water Science, Faculty of Agriculture,
Alexandria University, Postal code 21545 El-Shatby, Alexandria, Egypt.
E-mail: azzakassem82@gmail.com
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Soil salinity is one of the major environmental challenges that adversely affect crop productivity worldwide, especially in arid and semi-arid regions. The accumulation of dissolved salts, particularly sodium chloride (NaCl), leads to osmotic stress and ionic toxicity, which reduce the ability of plants to absorb water and essential nutrients (Zhang *et al.,* 2019 and Dar *et al.,* 2020).

Most importantly, these adverse conditions disrupt the microbial balance in the soil and inhibit the metabolic activity of beneficial microorganisms, including nitrogen-fixing bacteria like *Azotobacter*. At the physiological level, high salt levels lead to a decrease in soil water potential, making it a difficult environment for survival. Microbes suffer from osmotic stress, which disrupts cellular functions, and ionic toxicity, which damages cell membranes and inhibits the activity of essential enzymes (Usha & Kanimozhi, 2011 and Ashraf *et al.,* 2019).

Despite this, some strains of *Azotobacter* show remarkable resilience to saline environments, which is due to adaptive mechanisms such as the accumulation of compatible solutes (e.g., proline) and the production of stress-responsive enzymes. These mechanisms enable the bacteria to maintain their biological activity even under high salt concentrations (Nautiyal *et al.,* 2013; Astafyeva & Shalabayeva, 2016 and Abdel Latef *et al.,* 2020).

In addition to fixing atmospheric nitrogen, these bacteria play a multifaceted role in promoting plant growth. They produce plant growth hormones such as indole-3-acetic acid (IAA), gibberellins, and cytokinins, which stimulate root development and increase plant tolerance to environmental stresses (Rashed *et al.,* 2017). They also produce exopolysaccharides (EPS) that improve soil structure and water retention, creating a more favorable environment for plant roots and microbial communities (Abdel Latef *et al.,* 2021 and Sharma *et al.,* 2020).

Integrated strategies and the importance of soil respiration:

Recent studies have shown that the integration of *Azotobacter* with organic amendments such as vermicompost enhances microbial survival and CO_2 emissions in saline soils (Paul *et al.,* 2021). The role of vermicompost is not limited to providing essential nutrients, but it also acts as a stable source of carbon, improves soil structure, and mitigates salinity stress (Rietz and Haynes, 2003).

This dual strategy, which combines beneficial microorganisms with organic materials, is an integrated approach to addressing the biological degradation of saline soil. Soil respiration, measured by the emission of carbon dioxide (CO_2), is a primary and vital indicator of soil health and microbial activity (Verstraete, 1982 and Raich & Schlesinger, 1992).

It reflects the intensity of organic matter mineralization and microbial metabolism, providing a reliable measure of the biological response to stress conditions such as salinity (Kaur *et al.,* 2010 and Sakin & Yanardağ, 2019).

Study aim:

In light of these considerations, this study aims to identify *Azotobacter* strain isolated from naturally saline soil in the El-Moghraa area of Matrouh, Egypt, and to evaluate its ability to tolerate salinity.

Furthermore, the study measures the effect of introducing this bacterium, in combination with chemical fertilizers and vermicompost, on soil respiration at two levels of salinity.

Through this approach, we seek to provide a scientific basis for using these microorganisms as effective and sustainable biofertilizers, especially when combined with organic soil conditioners, to address the critical problem of salinity in agricultural systems.

MATERIALS AND METHODS

Soil samples were collected from Elmoghraa area of Matrouh, Egypt from a depth of 0–30 cm, air-dried, passed through a 2 mm sieve, and stored in plastic bags for the study, experiments, and analysis. The particle size percentage of the soil was determined to identify soil texture using the hydrometer method (FAO, 1974). The pH and electrical conductivity (EC) were measured in soil paste extracts according to Page *et al.* (1982). Total carbonates were determined by the calcimeter method (Nelson and Winter, 1982). Total organic carbon in the soil was determined by wet-oxidation according to the Walkley-Black method (Page *et al.,* 1982 and Verstraete, 1982). The soil characteristics are shown in Table 1.

1. Isolation and Identification of Salt-Tolerant *Azotobacter*

Azotobacter spp. were isolated using Ashby's nitrogen-free medium (Akhter *et al.,* 2012 and Soniari & Atmaja, 2019). Colonies were purified. Salt tolerance was assessed using sodium chloride (NaCl)-enriched media (Singh & Jha, 2016 and Usha & Kanimozhi, 2011).

Table 1. Physicochemical Properties of the Studied Soil Surface Layer (0–30 cm)

Soil characteristics	
pH	7.81
EC (dSm^{-1}) *	14.0
Total $CaCO_3$ (%)	0.61
TOC (%)	0.26

Particle size distribution (%)	
Sand	78
Silt	9
Clay	13
Soil texture	Loamy sand

*Saturated soil paste extract.

2. Molecular Characterization of *Azotobacter* spp. Isolates

DNA Extraction and 16S rRNA-Based Molecular Identification

Genomic DNA was extracted from *Azotobacter* isolates cultured overnight in LB broth (28 °C, 200 rpm), following the CTAB method (Sambrook and Russell, 2001). The 16S rRNA gene was amplified by PCR using universal primers (Table 2) SSU2F and SSU1492R in a 25 µL reaction mix. The amplification conditions included an initial denaturation at 94 °C (30 s), followed by 35 cycles of 94 °C (30 s), 55 °C (1 min), and 72 °C (90 s), with a final extension at 72 °C (10 min). The amplified products were visualized on a 1% agarose gel. Purified PCR products were sequenced bidirectionally (Macrogen, Korea). The sequence quality was assessed using Chromas, and the consensus sequences were analyzed via BLASTn for species identification (Lane, 1991).

3. Effect of Sodium Chloride (NaCl) on *Azotobacter*

Different concentrations (0%, 1%, 2%, 3%, 4%,5%, 6%,7% 8%,9% and 10%) of sodium chloride (NaCl) were added to Ashby's nitrogen-free medium (Usha & Kanimozhi, 2011; Akhter *et al.,* 2012 and Astafyeva & Shalabayeva, 2016). 100 ml of each medium was added to each flask. These media were sterilized in an autoclave at 121 °C for 15 minutes. Three flasks were inoculated and incubated for 4 days at 30 °C. Bacterial cultures were measured using a spectrophotometer (Voltage 230/115v, Frequency 50/60Hz Power 13VA Model 631) at 620 nm (Usha and Kanimozhi, 2011).

4. Incubation Experiment

In a 1000 ml bottle, 200 g of soil and seven treatments were added. After determining the volume of water needed to achieve 60% of the soil's water holding capacity (WHC), a cover was placed on each bottle. The samples were incubated at 28 °C for 3, 7, 14, 21, and 28 days. At the end of each period, soil respiration was estimated (Verstraete, 1982 and Raich & Schlesinger, 1992). At the end of the experiment, pH, EC, and the number of *Azotobacter* sp. were estimated.

Seven treatments:

1-T1: Control (EC 6.8)

T2: EC 14 + *Azotobacter*

T3: EC 14 + Steralized soil + *Azotobacter*

T4: EC 14 + *Azotobacter* + Vermicompost

T5: EC 6.8 + *Azotobacter*

T6: EC 6.8 +Steralized soil + *Azotobacter*

T7: EC 6.8 + *Azotobacter* + Vermicompost

5. Measurement of Soil Respiration

Carbon dioxide (CO_2) evolution was measured using the alkali absorption method (Verstraete, 1982). The quantity was determined by titration with hydrochloric acid (HCl) and expressed in units of mg C-CO_2 kg^{-1} soil per day (Kaur *et al.,* 2010 and Sakin & Yanardağ, 2019).

6. Data Analysis

The data were analyzed using one-way analysis of variance (ANOVA) and LSD tests at a $P < 0.05$ level (Rietz and Haynes, 2003).

RESULTS AND DISCUSSION

1. Molecular Identification of the *Azotobacter chroococcum* Strain and its Salinity Tolerance

Successful amplification of the 16S rRNA gene using universal primers SSU2F and SSU1492R yielded a PCR product of approximately 1500 base pairs (Lane, 1991 and Sambrook & Russell, 2001). The purified amplified products were subjected to bidirectional Sanger sequencing. BLASTn analysis of the consensus sequence revealed a high degree of similarity (100%) with *Azotobacter* chroococcum sequences available in the NCBI GenBank database. The obtained sequence was deposited in GenBank under accession number PV489975, ensuring its accessibility for future comparative and phylogenetic studies.

Table 2. 16S rRNA Primers Used for the Molecular Identification of *Azotobacter* Isolates

Gene name	Primer name	Primer sequence (5'→3')	Reference
16S rRNA	SSU2F	AGAGTTTGATCMTGGCTCAG	Lane (1991)
	SSU1492 R	TACGGYTACCTTGTTACGACTT	

These molecular findings confirm the taxonomic identity of the isolate as *Azotobacter chroococcum* and provide a reliable basis for further research into its functional characteristics and potential biofertilizer applications. This step is essential to ensure the accuracy and reproducibility of the research. The strain's

ability to grow in media containing high concentrations of sodium chloride (NaCl) demonstrates significant salinity tolerance (Usha & Kanimozhi, 2011 and Dar *et al.*, 2020), confirming that it is a strain capable of adapting to the natural saline environment from which it was isolated. This ecological adaptation is a fundamental principle in microbial ecology and makes this strain a promising candidate for agricultural applications in similar soils. The results indicate that the optical density of the bacterial cultures decreased with increasing sodium chloride (NaCl) concentration but remained measurable, which indicates robust adaptive mechanisms that allow the bacteria to maintain metabolic activity even under stressful conditions (Nautiyal *et al.*, 2013 and Ashraf *et al.*, 2019).

2. Effect of Treatments on Soil Respiration

Azotobacter inoculation enhanced soil respiration under both EC 14 and EC 6.8 conditions (Sharma *et al.*, 2020). CO_2 emissions increased notably with the addition of vermicompost (Paul *et al.*, 2021). Even under EC 14 conditions, *Azotobacter* maintained its activity, suggesting strong salinity tolerance and adaptability. The study provides quantitative evidence that microbial activity, measured by soil respiration, was significantly improved in all treatments containing *Azotobacter* compared to the control. The data, which represents the rates of CO_2 emission for each treatment over the incubation period, highlight the critical role of organic additives in enhancing this activity (Rietz & Haynes, 2003 and Zhang *et al.*, 2019).

Soil respiration was significantly higher in all treatments inoculated with *Azotobacter* compared to the control (T1), indicating that the bacterium successfully colonized the soil and maintained its metabolic activity despite salinity stress (Dar *et al.*, 2020 and Sharma *et al.*, 2020). The highest respiration rates were observed in treatments with vermicompost (T4 and T7), confirming the synergistic effect of the microbial inoculant and the organic amendment (Rietz & Haynes, 2003; Zhang *et al.*, 2019; Dar *et al.*, 2020 and Paul *et al.*, 2021). Vermicompost provides a protected microenvironment for the bacteria by supplying essential carbon and nutrients and mitigating osmotic and ionic stress (Rietz & Haynes, 2003; Zhang *et al.*, 2019 and Paul *et al.*, 2021). Although the *Azotobacter* strain is salt-tolerant, its metabolic activity is more efficient under less severe salinity conditions, as evidenced by the higher respiration rates in the EC 6.8 treatments compared to the EC 14 treatments (Nautiyal *et al.*, 2013; Ashraf *et al.*, 2019 and Abdel Latef *et al.*, 2020). This finding suggests that the biofertilizer's effectiveness is maximized when integrated with other management practices aimed at reducing soil salinity (Abdel Latef *et al.*, 2020).

Fig. 1, depicts the cumulative CO_2 production over a 28-day incubation period. This represents the total microbial respiration in the soil for each treatment group.

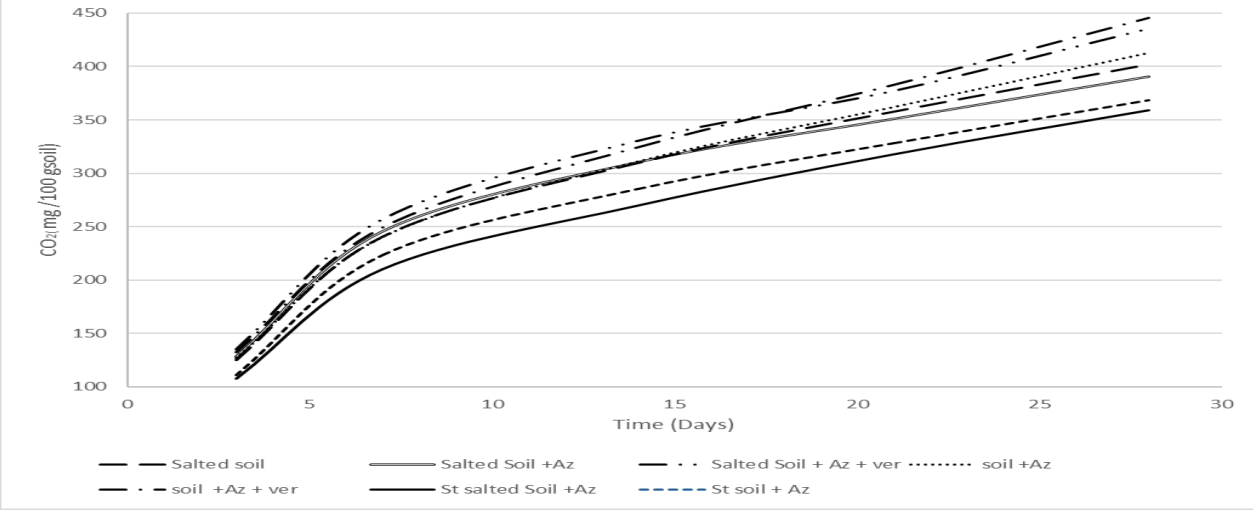

Fig. 1. Cumulative CO_2 Production Over Time

The treatments with vermicompost ("Salted Soil + Az + ver" and "soil + Az + ver") show the highest cumulative CO_2 production. Soil + Az + ver has the highest value, followed closely by the salted Soil + Az + ver. This indicates that adding vermicompost significantly boosts overall microbial activity.

The treatments with *Azotobacter* but no vermicompost ("Salted Soil + Az" and "soil + Az") show a moderate increase in cumulative CO_2 production compared to the control group.

The control groups, represented by "Salted soil" (the light blue line) and "St salted Soil + Az" and "St Soil + Az", have the lowest cumulative CO_2 production. This suggests that without the organic amendments or *Azotobacter*, microbial activity is limited.

The curves for all treatments rise steeply in the first 7-10 days, then the rate of increase slows down. This is expected, as the most easily decomposable organic matter is consumed quickly at the beginning of the experiment.

Fig. 2, shows the rate of CO_2 production per day over the 28-day incubation period. This represents the instantaneous microbial activity.

All treatments show a high initial rate of CO_2 production, which then declines over the 28-day period. This is a typical pattern for soil respiration experiments where an organic amendment is added, as the easily available carbon is consumed first.

Similar to the cumulative production graph, the treatments with vermicompost have the highest initial respiration rates ("Salted Soil + Az + ver" and "soil + Az + ver"). The "soil + Az + ver" line starts with the highest rate, indicating the strongest initial microbial response.

The decline in respiration rate over time suggests that the microbial population is adjusting to the available resources, and the readily available food source is being depleted.

The lines for all treatments seem to converge towards the end of the experiment, indicating that the differences in daily respiration rates become smaller as the experiment progresses.

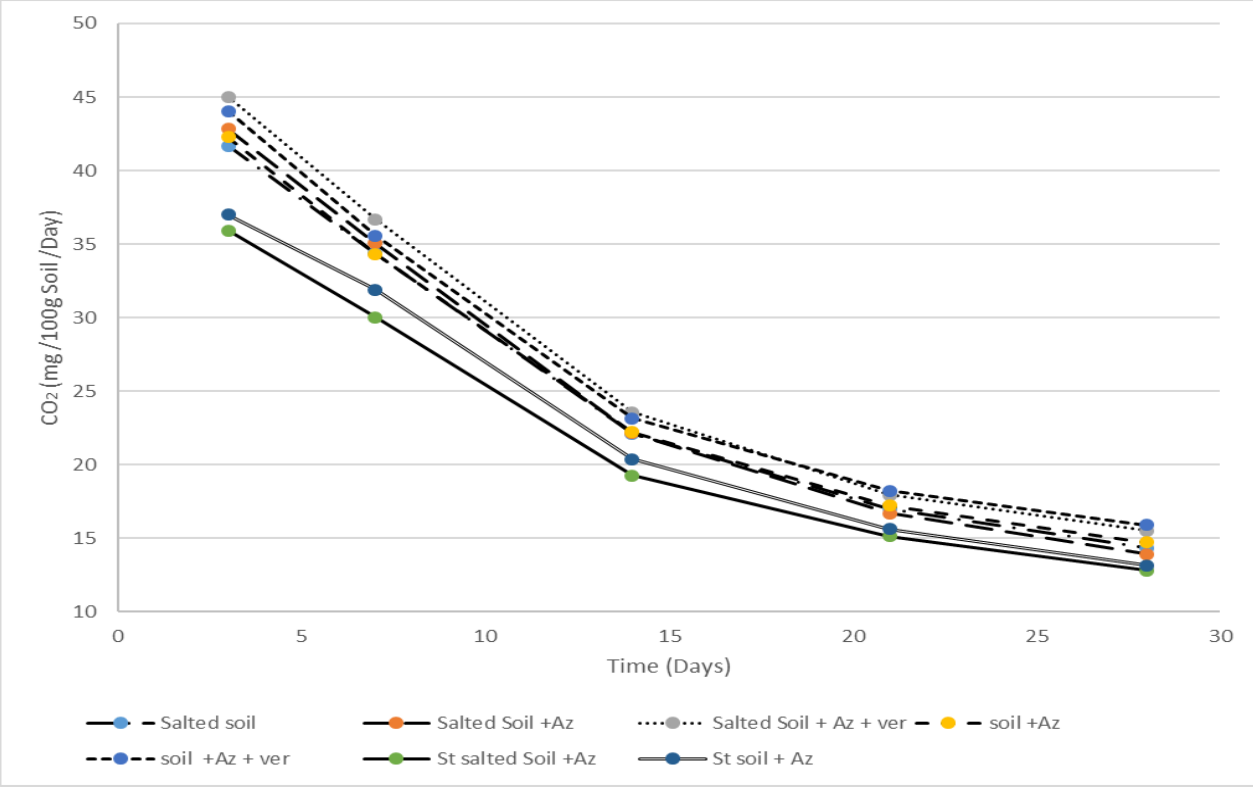

Fig. 2. Rate of CO_2 Production Over Time

In summary, Fig. 1 shows the total effect of the treatments, highlighting the long-term impact on overall microbial activity. Fig. 2 provides a more detailed look at the short-term dynamics of soil respiration, showing that the treatments cause a rapid and significant increase in microbial activity, which then gradually slows down as the available resources are consumed. Both figures confirm that the addition of vermicompost has the most significant positive impact on soil respiration.

The data clearly show that soil respiration was significantly higher in treatments containing vermicompost (T4 and T7) compared to the other treatments. This result is attributed to the fact that vermicompost not only acts as a nutritional additive but also provides a rich and stable source of carbon and nutrients that are essential for the chemoorganotrophic *Azotobacter chroococcum* (Rietz & Haynes, 2003 and Paul *et al.,* 2021). Furthermore, vermicompost improves soil structure and increases its water-holding capacity, which mitigates the effects of osmotic and ionic stress caused by salinity (Dar *et al.,* 2020). This addition provides a protected microenvironment for growth and survival, which explains the significant increase in metabolic activity compared to treatments that do not contain organic matter (Zhang *et al.,* 2019). Soil respiration is a reliable indicator of soil health and microbial activity (Verstraete, 1982 and Raich & Schlesinger, 1992). This study demonstrates a direct causal relationship between the application of *Azotobacter* and organic additives and the enhancement of microbial activity in soil under salinity stress conditions. A comparison between the different salinity groups (EC 6.8 and EC 14) indicates that microbial activity was generally higher at the lower salinity level (treatments T5, T6, and T7). Although the isolated *Azotobacter* strain shows remarkable tolerance to high salinity (EC 14), its metabolic activity remains more efficient under less stressful conditions (Nautiyal *et al.,* 2013; Ashraf *et al.,* 2019 and Abdel Latef *et al.,* 2020). This observation enhances our understanding of the limits within which even stress-tolerant microorganisms operate and provides practical insights into how management strategies can be adapted based on the severity of soil salinity.

3. Synergistic Effect of Amendments and Bio-inoculants: A Comprehensive Perspective

Integrating the results of this study with recent research enhances a deeper understanding of how to sustainably improve the health of saline soil. Our results provide strong evidence that the use of *Azotobacter chroococcum* as a biofertilizer is not only effective due to its ability to fix nitrogen and produce growth hormones (Wani *et al.,* 2013; Chennappa *et al.,* 2017 and Romero-Perdomo *et al.,* 2017) but also because of its ability to stimulate overall microbial activity under stressful conditions (Ashraf *et al.,* 2019 and Sharma *et al.,* 2020). Other studies confirm that *Azotobacter chroococcum* enhances crop growth and reduces the need for chemical fertilizers, which supports the conclusions of this study (Mahajan *et al.,* 2003 and Tawfik *et al.,* 2011).

The success of treatments that combine bio-inoculants and organic additives represents a paradigm shift from solutions that rely on individual inputs to agricultural management based on integrated systems (Sumbul *et al.,* 2020). The organic addition of vermicompost does not just provide nutrients; it acts as a natural buffer against salt stress, allowing beneficial bacteria to thrive (Paul *et al.,* 2021). This synergistic relationship creates a positive feedback loop: vermicompost improves the physical and chemical environment of the soil, while *Azotobacter* bacteria enhance the soil's biological functions, leading to a more resilient and sustainable system (Rietz & Haynes, 2003 and Dar *et al.,* 2020). Table 3 illustrates this synergistic effect and its broader implications for soil health.

Effect of salinity on pH, EC and count of *Azotobacter*

Fig. 3, is a bar chart that displays the final soil pH and electrical conductivity (EC) for different treatments.

Soil pH: The graph shows that the pH values are within a similar, slightly alkaline range for all treatments, with a few notable exceptions. The treatment "Soil + Az + ver" (soil with *Azotobacter* and vermicompost) has the lowest pH. This is a significant finding because vermicompost can help to neutralize alkaline soils by adding humic and fulvic acids, which can improve nutrient availability.

Electrical Conductivity (EC): The EC values, which measure salinity, show a clear separation between the two main soil types. The treatments on the left ("Soil" and "Soil + Az") have a low EC, indicating low salinity. In contrast, the treatments on the right ("Salted Soil" and "Salted Soil + Az") have a much higher EC, confirming their high-salinity status. The addition of vermicompost in the "Salted Soil + Az + ver" treatment appears to have a slight mitigating effect on EC compared to the "Salted Soil + Az" treatment, suggesting that it helps to reduce the overall salt concentration. This is a key benefit of using organic amendments in saline soils.

Fig. 4, is a bar chart showing the count of *Azotobacter* colonies in the soil for each treatment.

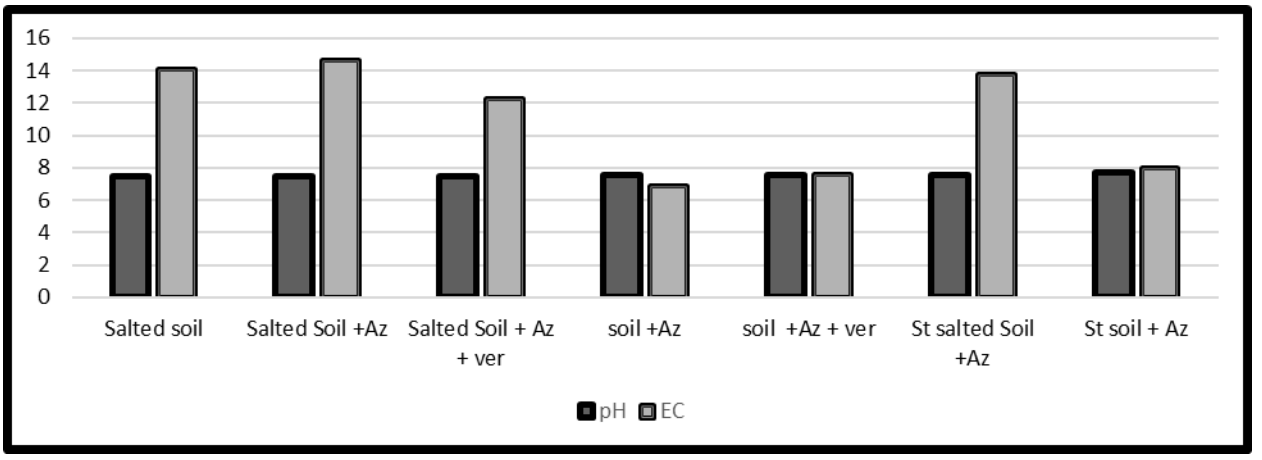

Fig . 3. Effect of Treatments on Soil pH and EC

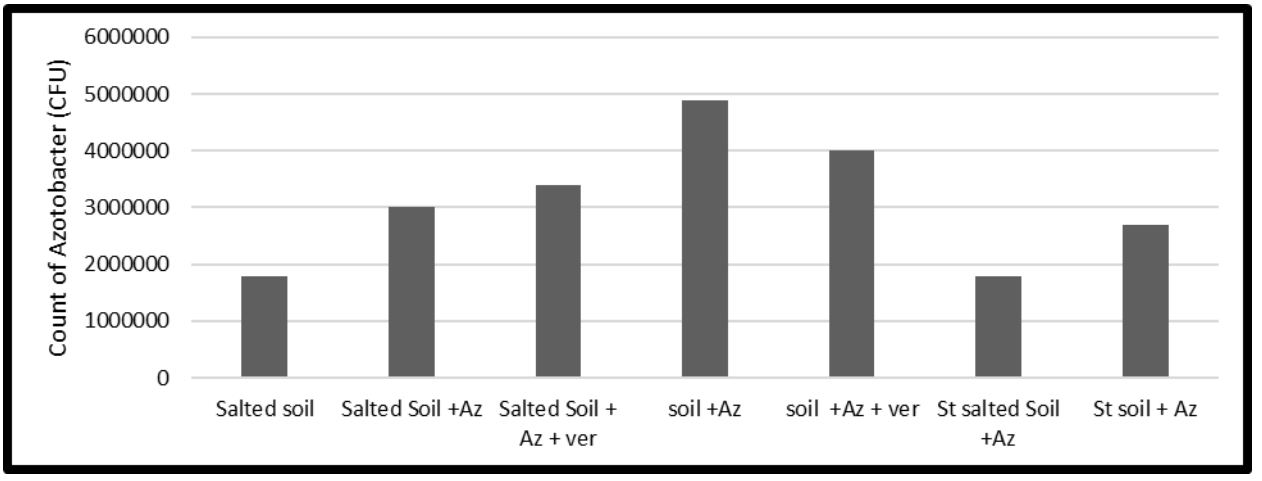

Fig. 4. Effect of Treatments on *Azotobacter* Count

Impact of *Azotobacter* and Vermicompost: The treatments inoculated with *Azotobacter* have a higher colony count than the un-inoculated control treatments. This is expected and shows that the bacteria were successfully introduced into the soil.

Synergistic Effect of Vermicompost: The most notable result is the significant increase in the *Azotobacter* population when vermicompost is added. The "Salted Soil + Az + ver" treatment has a much higher colony count than the "Salted Soil + Az" treatment. This confirms that vermicompost creates a more favorable environment for the bacteria to thrive, even under saline stress. This is likely due to the organic matter, nutrients, and improved soil structure provided by the vermicompost, which helps the bacteria tolerate the harsh conditions of high salinity.

Salinity Stress: While the addition of *Azotobacter* to the saline soil ("Salted Soil + Az") increases the count compared to the control, the number of colonies is lower than in the non-saline soil with *Azotobacter* ("Soil + Az"). This demonstrates that while the isolated *Azotobacter* strain is salt-tolerant, its growth and proliferation are still negatively affected by high salinity. This aligns with a known negative correlation between soil EC and *Azotobacter* populations.

In conclusion, the figures clearly illustrate that salinity negatively impacts soil properties and the population of *Azotobacter*. However, the application of vermicompost acts as an effective amendment, improving the soil environment by slightly reducing salinity and, most importantly, significantly boosting the count and survival of the inoculated *Azotobacter* strain, thus mitigating the negative effects of salinity.

Salinity Tolerance of Isolated *Azotobacter*

The effect of varying concentrations of sodium chloride (NaCl) on the growth of the isolated *Azotobacter chroococcum* strain was assessed by measuring the optical density (OD) of the bacterial

cultures at 620 nm (Usha and Kanimozhi, 2011). The results, as shown in Figure 5, demonstrate that the bacterium maintained measurable growth even at high concentrations of NaCl, although there was a clear trend of decreasing OD as salinity increased. The strain exhibited its highest growth at 0% NaCl concentration, with an OD of 1.25. At a concentration of 10% NaCl, the OD decreased to 0.45, confirming its significant salt tolerance and adaptive mechanisms (Usha & Kanimozhi, 2011; Nautiyal *et al.*, 2013 and Ashraf *et al.*, 2019). This finding is consistent with the results of the soil respiration experiment, which showed that the strain remained

Fig. 5, shows the effect of different sodium chloride (NaCl) concentrations on the growth of the *Azotobacter* chroococcum strain, as measured by absorbance (A) at 620 nm.

The figure demonstrates that the growth of *Azotobacter* is directly influenced by salinity. As the NaCl concentration increases, the absorbance initially rises, peaking at a salinity of 6%. This indicates that the bacterium is thriving at this salt concentration. After the 6% peak, the absorbance begins to decline as salinity continues to rise, reaching its lowest point at the highest concentration of 10% NaCl.

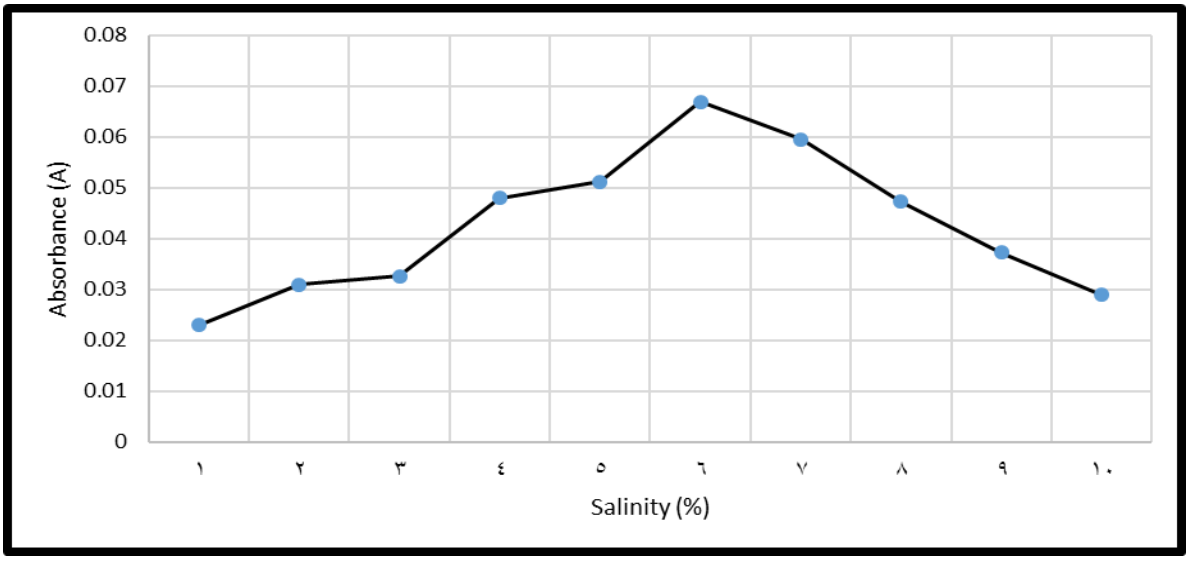

Fig. 5. Effect of Sodium Chloride (NaCl) on the Growth of *Azotobacter chroococcum*

Table 3. Summary of Key Findings and Their Implications for Sustainable Agriculture

Key Result	Underlying Mechanism	Broader Implications
A native *Azotobacter* strain was identified as *A. chroococcum*	Accurate molecular characterization using 16S rRNA gene sequencing	A reliable basis for developing biofertilizers specifically designed for saline regions
Increased soil respiration with *Azotobacter* inoculation	Increased microbial metabolic activity and activation of the native microbial community	Demonstrates the effectiveness of *Azotobacter* as a catalyst for biological activity under salinity stress
Highest soil respiration rates with vermicompost addition	Vermicompost provides a stable source of carbon and nutrients and mitigates osmotic stress	Confirms the critical role of organic amendments as a protective buffer to enhance the survival of bio-inoculants
Synergistic effect of bio-inoculant and vermicompost	Combining microbial stimulation with protection of the soil's microenvironment	Provides a model for sustainable agriculture that improves soil resilience through an integrated approach

This data confirms that the isolated *Azotobacter* strain is salt-tolerant. Its ability to maintain measurable growth

even at high salt concentrations (up to 10%) suggests it possesses robust adaptive mechanisms to withstand stressful conditions.

This finding is consistent with the results of the soil respiration experiment, which showed that the strain remained metabolically active under high-salinity conditions. The figure provides a direct visual representation of the strain's physiological response to salinity, reinforcing the study's conclusions about its viability as a biofertilizer in saline soils.

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that a native *Azotobacter* strain, isolated from saline soil (EC 7.5 dS/m) in Elmoghraa area of Matrouh, Egypt, exhibited high tolerance to salinity and retained strong metabolic activity, as evidenced by CO_2 emissions in the soil (Abdel Latef *et al.,* 2020 and Dar *et al.,* 2020). The bacterium not only survived but also remained functionally active under elevated salinity levels (EC 12), especially when supported by organic amendments like vermicompost (Rietz & Haynes, 2003 and Paul *et al.,* 2021). *Azotobacter* is a viable biofertilizer for saline soils (Mahajan *et al.,* 2003; Tawfik *et al.,* 2011 and Romero-Perdomo *et al.,* 2017), CO_2 emission is an effective indicator of microbial performance (Verstraete, 1982 and Raich & Schlesinger, 1992), and organic amendments improve microbial resilience in salt-affected soils (Zhang *et al.,* 2019).

This study summarizes several key conclusions. First, it successfully demonstrated that a native *Azotobacter chroococcum* strain, isolated from saline soil, possesses a high capacity for adaptation and salinity tolerance (Usha & Kanimozhi, 2011 and Astafyeva & Shalabayeva, 2016). Second, it confirmed that soil respiration is a reliable indicator for assessing microbial activity and the effectiveness of treatments under stressful conditions (Sakin and Yanardağ, 2019). Third, the results unequivocally showed that the combination of bio-inoculants and organic amendments, such as vermicompost, generates a synergistic effect that enhances the biological activity of the soil, surpassing the use of either alone (Sumbul *et al.,* 2020). These conclusions provide a strong scientific framework for developing sustainable solutions for agriculture in salinity-affected environments.

Based on these results, the study provides the following recommendations:

- Use salt-tolerant *Azotobacter* strains as part of farming programs in salinity-affected soils (Wani *et al.,* 2013).
- Integrate microbial inoculants with organic amendments such as vermicompost to increase their effectiveness. This approach offers an integrated solution that combines biological and physical benefits (Sumbul *et al.,* 2020).
- Conduct additional genomic and transcriptomic analyses on the salt-tolerant *Azotobacter* strain to discover the specific genes and proteins responsible for the salinity tolerance mechanisms. This research could accelerate the process of genetic engineering to improve future strains (Singh and Jha, 2016).
- Develop commercial biofertilizer formulations specifically designed for salinity-affected regions, combining the microbial inoculant with an organic conditioner in a single product.
- Conduct large-scale field experiments to validate these results under real environmental conditions and monitor their long-term effects on soil health and crop productivity.
- Analyze the response of this salt-tolerant strain to other combined abiotic stresses, such as salinity and drought or heavy metal pollution.

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الملخص العربي

عزل والتعرف علي سلالة *Azotobacter chroococcum* وقياس التنفس الميكروبي في الأرض الملحية

عزة مسعود

بكتيريا الأزوتوباكتر كائنات حية دقيقة مثبتة للنيتروجين الجوى لا تكافليا وتُنتج هرمونات نباتية، مما يُسهم في خصوبة الأرض ونمو النبات. ومن المعروف أن ملوحة الأرض تُسبب اضطرابات كثيرة في النظام البيئي الميكروبي مما يؤثر على الوظائف البيولوجية للبكتيريا النافعة فى الأرض. كما أن للظروف الملحية وخاصةً التركيزات العالية من كلوريد الصوديوم أن تُثبط نمو هذه البكتيريا ونشاطها الأيضي. هذه الدراسة توضح قدرة بكتيريا الآزوتوباكتر للبقاء ونشاطها في الأرض الملحية. ولذا تُقيّم هذه الدراسة تنفس الأرض المُقاس بانبعاثات ثاني أكسيد الكربون كمؤشر رئيسي للنشاط الميكروبي في ظل ملوحة الأرض العالية. ومن خلال عزل وتوصيف سلاله الآزوتوباكتر من أرض منطقه المغرة وإضافتها إلى أرض شديدة الملوحة وقياس نشاطها الميكروبي كانت السلالة قادرة على تحمل ظروف ملوحة الأرض القاسية. وأظهرت النتائج النشاط البيولوجي لبكتيريا الآزوتوباكتر في الأرض موضحة إمكانية استخدامها كأسمدة حيوية في النظم الزراعية الملحية. يُعد هذا البحث تقييمًا شاملاً لدور سلالة *Azotobacter chroococcum* التي تم تأكيد هويتها من خلال تسلسل جين 16 S rRNA في تحسين صحة الأرض الملحية. كما أوضحت النتائج أن البكتيريا تزيد من تنفس الأرض حتى في ظل ظروف الملوحة العالية (EC = 14 dS/m) مما يؤكد قدرتها الكبيرة على تحمل الملوحة. والأهم من ذلك، أن إضافة المادة العضوية على شكل سماد دودي عززت هذا التأثير بشكل كبير. حيث يوفر السماد الدودي مصدر الكربون والمغذيات للبكتريا بالإضافة إلى دوره في تخفيف الإجهاد الأسموزي والأيوني الناتج عن الملوحة مما يخلق بيئة محمية مناسبة لزيادة النشاط الميكروبي. هذه الدراسة تقدم أستراتيجية قوية قائمة على الأدلة للإدارة المستدامة للأرض في المناطق المتأثرة بالملوحة، مما يوفر بديلاً صديقًا للبيئة بدلا من المدخلات الكيميائية التقليدية في الزراعة.

الكلمات المفتاحية: الأزوتوباكتر، الأرض الملحية، تنفس الأرض، كلوريد الصوديوم ($NaCl$)، النشاط الميكروبي، انبعاث ثاني أكسيد الكربون (CO_2)، الأسمدة الحيوية.