EMPLOYMENT OF NANOBIO COMPOSITE TO SUPPORT PLANT GROWTH UNDER SALINE CONDITION

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A thesis submitted to Bahria University, Islamabad in partial fulfillment of the requirement for the degree of MS in Environmental Sciences.

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DEDICATION

Dedicated to my parents, siblings, and friends, their tremendous support and cooperation kept me motivated and helped me throughout my thesis year.

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With the grace of Almighty ALLAH, I have overcome another impossible task with ALLAH’s blessings.

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ABSTRACT

This study explores the impact of soil salinity on agricultural productivity and investigates the use of ZnO NPs and biochar to alleviate salt stress in saline soils. Unlike gypsum, ZnO NPs offer more plant benefits, providing essential micronutrients and nutrient uptake Biochar outperforms gypsum in improving soil health and water retention and is more sustainable. ZnO nanoparticles were synthesized using *Corriandrum sativum* extract and characterized using SEM, XRD, and FTIR. The SEM-EDS analysis revealed ZnO NPs have a mesoporous structure and a high surface area, making them highly reactive and suitable for alleviating salt stress. The EDS analysis indicated that NPs consist of 40% zinc, 34% oxygen, and 24% carbon. The XRD analysis confirmed the crystalline structure of the nanoparticles, showing distinct peak planes of ZnO, indicating a hexagonal wurtzite structure and their high crystallinity. The FTIR analysis found characteristic Zn-O bond peaks at 689.32 and 430.14 cm^{-1}, confirming the presence of Zn-O bonds and the formation of ZnO nanoparticles. The plant maize (*Zea mays*) was used to check the antioxidant response to treatments and examined the effects of treatments with ZnO NPs, BC, and nano biocomposite on soil pH , EC, and nutrient content. Applying ZnO NPs reduced soil pH, from 8.61 to 7.58 in S1 and 9.67 to 7.25 in S2. ZnO NPs + BC treatment, the reduction in EC was significant, from 11.55 to 8.65 dS/m in S2. Nutrient levels also showed positive changes. Nitrogen content increased notably, in the P+NP from 11.83 to 64.66 mg/kg in S1. Potassium increased to 0.109 mg/kg and phosphorus up to 67.6 mg/kg when ZnO NPs applied. Proline, a stress indicator, decreased from 0.55 µmol/g FW to 0.17 in plants treated with ZnO NPs. CAT and POD activities increased significantly, indicating enhanced antioxidant defense. Overall, the research highlights the effectiveness of ZnO NPs, biochar, and composite in improving soil health, increasing nutrient availability, and mitigating salt stress, offering a promising solution for saline soil management.

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LIST OF ABBREVIATIONS

Acronyms	Explanation
SEM	Scanning electron microscopy
EDS	Electron dispersion X-ray spectroscopy
FTIR	Fourier transforms infrared spectroscopy
XRD	X-ray diffraction
S-1	Moderately saline soil
S-2	Highly saline soil
CAT	Catalase
POD	Peroxidase
NP	Nanoparticles
BC	Biochar

CHAPTER 1

INTRODUCTION

1.1 Salinity

Soil salinity, a widespread concern impacting agricultural yield and environmental well-being worldwide, has driven researchers to explore cutting-edge approaches to address this challenge (P. Sharma et al., 2021). One such promising avenue is the utilization of nanoparticles, which can potentially mitigate the detrimental effects of soil salinity (Gupta et al., 2023).

Recent climate change scenarios are expected to result in increased soil salinization because of rising sea levels and their effects on coastal areas, as well as temperature increases that will certainly cause increased evaporation and increased salinization. In dry and semi-arid environments, where soil salinity is usually high and precipitation may not be sufficient to prevent leaching, salinity is among the most significant abiotic stressors affecting crop yield. Numerous morphological, physiological, and biochemical processes such as plant development, seed germination, and water and nutrient uptake are impacted by salinity (Akbarimoghaddam et al., 2011)

1.1.1 Types of Salinity

There are two major sources for the occurrence of salinity in soil one of them is Natural salinity (primary salinity) in which salts build up over time in the soil and groundwater, resulting in natural salinity over time (Salinity and Water Stress,2009). Primary salinity is the result of two natural processes. One is the weathering parent material that contains soluble salts, such as NaCl. The other is salt deposition from the ocean, mostly NaCl, which is carried by wind and rain (Syed et al., 2021).

Secondly, there is the Human-induced salinity (secondary salinity) Secondary salinization is the outcome of anthropogenic actions. Among the most frequent reasons are barren land, using salt water to irrigate land, Seepage from canals, inadequate drainage, and Salts left behind on the soil's surface when saline water evaporates. Incorrect slope an excessive amount of irritation(Farid et al., 2018)

1.1.2 Indicators of salinity

Several indicators can be used to assess the salinity status of soils and water, including pH, electrical conductivity, and physical appearance. Soil’s pH, reflecting the acidity or alkalinity of a solution, tends to be higher in saline environments due to alkaline salts like sodium carbonate and sodium bicarbonate. While pH doesn't directly measure salinity, saline soils often feature higher pH levels due to the presence of sodium carbonate, leading to increased alkalinity.

In contrast, electrical conductivity directly measures a solution's ability to conduct an electric current, with higher values indicating elevated salinity levels is a measure of the acidity or alkalinity of a solution. In saline environments, the pH is typically higher due to alkaline salts, such as sodium carbonate and sodium bicarbonate(Pedrera-Parrilla et al., 2016). While pH itself is not a direct measure of

salinity, saline soils often have a higher pH due to the presence of sodium carbonate, which can make the soil more alkaline. Electrical conductivity, on the other hand, directly measures the ability of a solution to conduct an electric current, which is directly proportional to the concentration of dissolved salts (Corwin & Lesch, 2013). High electrical conductivity (EC) values indicate elevated salinity levels in soils. Soils are categorized based on their EC values as follows (Lin & Bañuuelos, 2015):

- Non-saline: 0–2 dS/m
- Slightly saline: 2–4 dS/m
- Moderately saline: 4–8 dS/m
- Strongly saline: >8 dS/m

As salinity in soil increases, the EC of the soil also increases significantly. It is a reliable indicator for determining the salinity levels(Pentoś et al., 2021). Saline soils and water bodies can be identified by their physical characteristics. Saline soils often display a white or gray crust on the surface, resulting from the accumulation of salts. Additionally, the presence of salt-tolerant vegetation, such as halophytes, can be indicative of saline conditions. Plants in saline soil often exhibit signs of stress, such as stunted growth, leaf burn, and wilting. Saline soil may appear more compact and have poor structure due to the high salt content(Arora & Dagar, 2019).

1.1.3 Salinity Effects on Nutrient Availability

The presence of high levels of salt in soil, known as soil salinity, has detrimental effects on plants. It leads to ion toxicity, osmotic stress, and deficiencies in key nutrients such as nitrogen (N), calcium (Ca), potassium (K), phosphorus (P), iron (Fe), and zinc (Zn). This inhibits water uptake from the soil and affects various stages of plant development such as germination, vegetative growth, and reproductive development(Ashraf et al., 2018). The salt in the water does not bind water to the soil more tightly, but it does cause plants to use more energy to extract the water they need. Excess salinity reduces the availability of water for plants and causes stress.

Salinity also interferes with nitrogen metabolism by reducing water availability and absorption, disrupting root membrane integrity, inhibiting nitrogen, altering the activities of nitrogen-assimilating enzymes, reducing transpiration, and decreasing the relative growth rate, leading to a lower nitrogen demand (Hussain et al., 2019).

High salinity in soil affects plants' availability and uptake of potassium (K^+). Excessive salts displace essential nutrients and reduce their availability in the soil solution, inhibiting nutrient uptake and impacting overall plant growth and health (Torabi et al., 2021). In most cases, salinity decreases phosphorus (P) concentration in plant tissue and intensifies adverse effects on phosphorus uptake. Additionally, in a high-saline environment, the movement of phosphorus from the root to the shoot is inhibited(Bouras et al., 2023).

1.1.4 Effects of Salinity on Plants

The plant’s defense mechanism activates when the stress condition is present the plant amino acid like proline and antioxidant enzymes acts as a coping mechanism to overcome stress and the effects caused by it. Under high salinity conditions, plants undergo significant changes in proline content. Proline serves as an antioxidant, helping to scavenge harmful reactive oxygen species (ROS) that are generated under salt stress, thereby protecting cellular structures and functions (Gharsallah et al., 2016).

These antioxidant enzymes play a crucial role in mitigating the oxidative stress induced by salinity, ensuring the protection and survival of plant cells under adverse conditions. Salinity stress also affects the activity of plants' antioxidant enzymes, such as catalase (CAT) and peroxidase (POD). Catalase activity typically increases under salinity stress, contributing to the breakdown of hydrogen peroxide (H_2O_2), a reactive oxygen species, into water and oxygen, thereby protecting cells from oxidative damage (Aazami et al., 2021). Similarly, peroxidase activity also increases in response to salinity stress. Peroxidases utilize H_2O_2 to oxidize various

substrates, aiding in detoxifying ROS and protecting cellular components. Additionally, peroxidases are involved in the lignification process, which strengthens cell walls and helps plants better withstand the mechanical stress caused by high salinity (Abdel Gawad et al., 2016).

1.2 Nanotechnology

The exceptional properties of nanoparticles, such as their minuscule size, high surface area-to-volume ratio, and tailored chemical reactivity allow substances to interact with each other. Soil components and plant systems in unique and innovative ways (Kuchanwar et al., 2022). Nano-enabled soil remediation techniques have shown promise in addressing the underlying causes of salinization, such as the collection of toxic ions and the depletion of key nutrients (Yadav et al., 2023).

It is anticipated that the applications of nanotechnology and nanoscience will have a major impact on sustainable development, affecting almost every industrial area, including information and communications technologies, healthcare, agrifood, transportation, energy, and materials. The first step in taking corrective action is being able to identify and measure the number of harmful compounds present in the environment, and nanotechnology can assist in creating better systems for monitoring the environment (Rickerby & Morrison, 2007).

1.2.1 Nanotechnology in Agriculture

Agricultural bioremediation aids in the resolution and restoration of the soil's natural state using sustainable remediation technology. It is an intriguing phenomenon when one considers how the interaction of nanoparticles can eliminate harmful elements from agricultural soil and restore its sustainability. This method has

demonstrated efficacy in managing agricultural resources, facilitating medicine delivery within plants, and preserving soil fertility (Prasad et al., 2017).

Using environmentally friendly remediation techniques, agricultural bioremediation helps resolve environmental issues and return soil to its original state. It's an interesting phenomenon to think about how hazardous elements can be removed from agricultural soil and turned into a sustainable resource through nano-nano interactions (Prasad et al., 2017). In addition to improving plant protection techniques and implementing nutrient efficiency as a novel key to increasing agricultural production, nanotechnology may offer practical answers to several issues facing the farming industry, including better crop varieties, protecting plants, disease detection, and plant growth monitoring.

1.2.2 Zinc oxide nanoparticles.

Nanoparticles have proved pronounced potential in increasing agricultural productivity through efficient use of contributions, advancing soil health, and offering solutions to agricultural and environmental issues. From the (Thounaojam et al., 2021) work demonstrated that they can be applied as nano fertilizers, nano pesticides, nano biosensors, and remediating contaminated soils. Additionally, applying NPs enhances the expression of stress-tolerant genes and proteins in plants, making them more resistant to abiotic and biotic stresses. The exceptional properties of NPs make them ideal for promoting sustainable agriculture.

Focused research has been conducted on Zinc Oxide (ZnO) nanoparticles because of their unique electrical, optical, mechanical, magnetic, and chemical properties that set them apart from their bulk counterparts (Parihar et al., 2018). In the current decade, ZnONPs have been used extensively in agricultural production. Zinc insufficiency has been identified to be among the primary issues in reducing agricultural output in soils with an alkaline composition. One of the most important minerals for plants is zinc. The primary component or cofactor of enzymes is zinc.

Zinc oxide nanoparticles, or ZnO NPs, are essential for germination, pollen function, fertilization, and the formation of chlorophyll (Ukidave & Ingale, 2022).

1.2.3 Zinc oxide nanoparticles applications.

Zinc oxide nanoparticles may increase food crop growth and yield. Different doses applied to seeds improved plant growth, germination, and seedling vigor. They also promoted stem and root development in seeds. (Parihar et al., 2018). Zinc oxide (ZnO) nanoparticles have been found to reduce salinity stress in common beans. Through a comprehensive examination covering physiological, biochemical, and nutritional parameters, it has been demonstrated that ZnO NPs are effective in mitigating the negative effects of salinity stress on bean plants. ZnO NPs can modify antioxidant defense mechanisms, thus reducing the severity of oxidative damage and promoting the growth of plants under salinity stress (Gupta et al., 2024).

ZnO NPs were effectively biologically synthesized using an *Ochradenus arabicus* shoot extract. When added to the in vitro culture media, these ZnO NPs considerably reduced the detrimental effects of NaCl in Salvia officinalis, particularly when the dose was 10 mg/L. This dosage enhanced the plant biomass, total chlorophyll, accumulation of proline, and activities of antioxidant enzymes in the plants exposed to 0, 75, 100, and 150 mM NaCl, suggesting that the function of the antioxidant system can be activated by ZnO NPs to reduce salt stress either by the adjustments of the biochemical parameters or by enzymes (Alenezi et al., 2022).

1.2.4 Effects of Salinity on Agricultural Land

Salt accumulation in soil, due to natural and human causes, poses a serious threat to the ecosystem, especially in semi-arid and arid regions worldwide. This endangers global agriculture and food supply, highlighting the need to quickly

identify salinity-affected areas to maintain land health and ensure a steady food source.(Haq et al., 2023).

Most crops, including vegetables, struggle to thrive in many parts of the world due to an excess of soluble salts in the soil, particularly in dry and semi-arid regions. Like other types of crops, Vegetable tolerance to salt varies substantially amongst crops. Some examples of vegetables that are highly sensitive to salt include cauliflower, broccoli, eggplant, tomato plants, potato, carrot, turnip, lettuce, cucumber, pepper, and pumpkin (EC less than 2.8 dS m−1). While peas, okra, carrots, and onions have an electrical conductivity level of 1.0–1.5 dS m−1, they are highly susceptible to salt. Red beet (*Beta vulgaris*) is thought to have a moderate tolerance to salt (Khondoker et al., 2023).

1.2.5 Salinity in Pakistan's Agricultural Land

Soil salinity has emerged as a significant challenge in Pakistan, adversely impacting the nation's agricultural productivity. As per recent estimates, nearly 10% of the world's total arable land is affected by soil salinity, and Pakistan is no exception (Syed et al., 2021). The arid and semi-arid regions of the country have been particularly vulnerable to the accumulation of salt in the soil, leading to a decline in crop yields and the conversion of once-productive farmland into saline lands (Alnusairi et al., 2021).

The issue of soil salinity in Pakistan can be attributed to both natural processes and human-induced factors. Natural processes such as the gradual weathering and erosion of rocks, the intrusion of seawater in coastal areas, and the high rates of evaporation in the arid climate, all contribute to the buildup of salts in the soil (Sagar et al., 2021). However, the problem has been exacerbated by human activities, such as the over-exploitation of groundwater for irrigation, the use of poor-quality water for crop production, and the lack of proper drainage systems to mitigate the accumulation of salts As a result of these factors, it is estimated that up to 50% of the irrigated land

in Pakistan has become saline, leading to a critical loss of yield profitability every year (Zahra et al., 2024).

1.3 Biochar

Pyrolysis is a process that involves heating organic compounds to produce biochar, a substance like charcoal. It has gained interest in agriculture for improving degraded soil and promoting plant growth by enhancing soil properties and enzymatic activities. In comparison to the control, plants grown in salinity without biochar had reduced chlorophyll content by 9.7%, 17%, and 30%. The addition of biochar improved chlorophyll levels. Under typical conditions, 2% biochar increased chlorophyll content by 4%, and 1% biochar increased it by 7% (Akhtar et al., 2015).Reclaiming salt-damaged soil with biochar as a soil amendment has been shown to improve croplands affected by salinity. This study aimed to determine how biochar soil amendment affected wheat under salinity stress in terms of germination, growth, and various physiological and biochemical parameters (Kanwal et al., 2018).

1.4 Problem statement

Salinity affects soil nutrient levels and crop growth. Gypsum is used in Pakistan to reduce salinity but doesn't offer the same benefits to plants as ZnO nanoparticles. ZnO NPs offer more benefits to plants, by providing essential micronutrients, enhancing antioxidant defense, and promoting nutrient uptake. Biochar outperforms gypsum in saline soils, improving soil health and water retention. Biochar is also more sustainable and environmentally friendly.

1.5 Research Objectives

1. The green synthesis of zinc-based nanoparticles and conducting a comprehensive characterization of synthesized nanoparticles.

2. Application of nano bio composite in saline soil to address its efficiency to improve plant biometrics and soil health.

3. To evaluate the impact of treatment on plant enzymes and antioxidants.

CHAPTER 2

LITERATURE REVIEW

2.1 Literature review

Salinity is a major issue in dry regions globally, affecting 800 million hectares worldwide and 6.3 million hectares in Pakistan. It leads to lower crop yields and loss of arable land. High salt concentrations in plant tissues can harm enzymes and cause oxidative stress. This makes it difficult for plant roots to absorb water and can be toxic to specific ions in the soil.(Shahzad et al., 2019).Further (Syed et al., 2021) In Pakistan, methods to reduce salinity include using chemical modifications like leaching and gypsum fertilizers, implementing bio saline agriculture technology, and planting non-conventional cereal crops.

The soil salinity level that affects plant growth depends on various factors, such as soil texture, salt composition, and plant species. The main source of salts in soils is the primary minerals present in soils and rocks within the Earth's crust. Through chemical weathering processes like hydrolysis, hydration, solution, oxidation, and carbonation, these salts are gradually released and become soluble(Zia-ur-Rehman et al., 2016). While the weathering of primary minerals serves as an indirect source for almost all soluble salts found in soils, saline soils typically form when sufficient salts accumulate from external sources brought by water flow. Soil

salinity is a common issue in areas that receive salt deposits from water bodies, such as oceans. This often occurs in low-lying coastal regions due to the accumulation of excess soluble salts adversely affecting plant growth and soil structure (Rengasamy, 2016).

Nanoparticles have shown great potential in increasing agricultural productivity through efficient use of inputs, improving soil health, and offering solutions to agricultural and environmental issues. From the (Thounaojam et al., 2021) work demonstrated that they can be used as nano fertilizers, nano pesticides, nano biosensors, and for remediating contaminated soils. Additionally, applying NPs enhances the expression of stress-tolerant genes and proteins in plants, thereby making them more resistant to biotic and abiotic stresses. The unique properties of NPs make them ideal for promoting sustainable agriculture.

NPs can enter a plant's system in several ways, but primarily through the roots and leaves (Etesami et al., 2021) Researched the efficiency of the nanoparticles following entry, NPs engage in cellular and subcellular interactions with plants, influencing morphological, physiological, biochemical, and molecular changes. The properties of the NPs and the type of plant involved will determine whether these interactions are favorable or negative. The impact of NPs on plant systems may depend on their chemical makeup, reactivity, size, and concentration in or on the plant. Different NPs can support salinity-stressed plant growth and development, according to the evidence currently available. Zn-NPs were applied topically to rapeseed (*Brassica napus L.*) plants under salinity stress in a different study it enhanced abiotic stress tolerance.

Research from (Türkoğlu et al., 2024) indicated that applying ZnO-NPs to plant leaves increases the activity of antioxidant enzymes like SOD, POD, and CAT. Salt stress in quinoa seedlings leads to higher levels of H2O2 and MDA which are indicators of oxidative stress. Applying Zn and ZnO-NPs to quinoa plants under salt stress increases the effectiveness of antioxidant enzymes, with ZnO-NPs appearing particularly beneficial. Additionally, applying ZnO-NPs under salt stress reduces MDA concentration, protecting cell membrane integrity and mitigating the

negative effects of oxidative stress. Similarly, ZnO-NP applications help counteract the effects of salt stress in *safflowers* and *okra plants*.

(Azim et al., 2022) work explored that protein and sugars are essential indicators of a plant's health. Proteins are thought to be the main source of nitrogen compounds that are connected to the growth of new plant tissue. The tomato seedlings treated with ZnONPs had significantly higher leaf protein and sugar content than the bulk Zn and control groups, by 23% and 5%, respectively, in addition to ZnO NPs. Increased accumulation of soluble sugars suggests a deregulation of the carbon flow, and the increase in pigment, protein, and sugar content may be largely attributed to an inducement in photosynthetic machinery. These findings are in line with a study that found that while ZnONPs at higher concentrations are phytotoxic to plants, they improved the sugar and protein content in tomato seedlings at lower concentrations.

Biomaterials and some nanocomposites are providing new hope to replace these chemical-based practices. These nano-bio products also provide good health to crops and soil microflora as well as shield against various pathogens. These nano-bioproducts interact with soil microbes and help in their enhanced populations which further helps in the proper growth of the crops. (Jha et al., 2024).The aim is to create an environmentally friendly, slow-release fertilizer using biochar at a nanoscale. This fertilizer will gradually release macro and micronutrients to crops and soil, reducing waste and pollution. It will also address issues with conventional fertilizers leaching, providing nutrients to plants for higher yields. (Lateef et al., 2019).

The plants' various growth parameters significantly improved because of the application of this nano-bio composite. Improved seed germination, total plant height, shoot height, root height, total fresh weight, shoot fresh weight, root fresh weight, total dry weight, shoot dry weight, root dry weight, number of branches, leaf area, number of pods, and length of pods were among these improvements. (Bhilkar et al., 2024). Research (Parkash & Singh, 2020) Indicated that the addition of biochar effectively reduced the negative impact of high salinity stress. Applying biochar to the soil improved the eggplant's physiological processes, as well as its root and shoot

growth, resulting in increased yield. The effects of both types of biochar on eggplant physiology, growth, and yield were similar.

From the research findings of (Singh et al., 2019). When wheat seeds were treated with green-synthesized ZnO nanoparticles instead of chemically synthesized ones, they showed longer roots and shoots. This is because the green-synthesized nanoparticles are smaller (35 nm) than the chemically synthesized ones (48 nm), allowing for increased zinc absorption. Similarly, peanut seeds treated with ZnO nanoparticles had a higher zinc absorption rate and better seedling growth compared to seeds treated with chelated zinc sulfate.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Sample area.

The soil sample was collected from Firoza, Rahim Yar Khan's district. The climate of that region is a warm desert which has less rainfall and more evaporation rates. The soil texture of that land varies from sandy loam to loam (Yar Khan et al., 2015). The salt-stressed soil sample was collected from the agricultural land. The capacity of the soil to hold the water is greater. However, salt makes it less cultivated for the plant’s growth. The research conducted focuses on two different types of soil collected from different locations. The other soil sample was collected from Islamabad. This soil is normal soil ideal for plant growth, later the soil is stimulated with salt stress. The achieved target of salinity was to reach the

conductivity of < 8 ds/m. This was done to assess the effectiveness of treatments in high-saline soil.

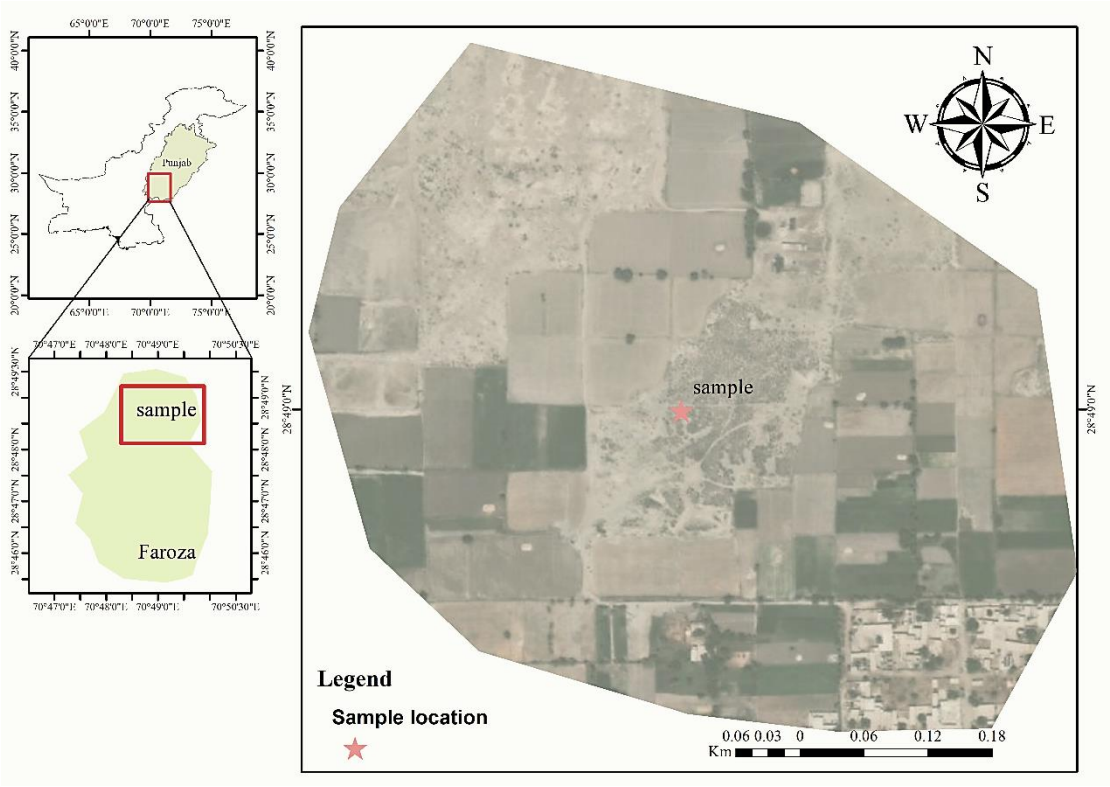

Figure 3.1 Map of soil sampling location S-1.

3.2 Experimental setup

The soil was of two types, one was already existing saline soil collected from an agricultural land barren due to salinity having moderate salinity to it. This soil was labeled as S1, and its EC varied from 4 ds/m to 5 ds/m which means it was moderately saline. Whereas there was another artificially made saline soil by adding salt into the soil. Its EC was set to make it highly saline by maintaining its electrical conductivity from 9ds/m to 10ds/m. Artificially made saline was labeled as S2. The 14 pots were applied with the treatments in total.

The treatment of ZnO nanoparticles and biochar was applied separately as well as in combination with both. The biochar was used with 2% to 100 g/soil, 2% by weight (Zheng et al., 2013). The nanoparticles ratio was calculated and applied 0.7g for 700g of soil and 0. 35 g for 350 g of soil (Adil et al., 2022). The readings were taken from the initial day 1- to the final reading. The readings are noted according to pre-treatment and post-treatment of the soil. Results are categorized and varied depending on the treatments and day relative to soil 1 and soil 2 separately.

Table 3.1 Experimental setup of treatment plan and analyses.

testing/plan span		**S-1**		**S2**
	Types of soil	*Moderately Saline (EC>4 to 5 ds/m)*		*Highly Saline (EC>8 to 10ds/m)*
Finalvalue_15days_10days_ Initial value		*S1 (Control) & S2(Control)*		
		S+NP	***S+BC***	***S+NP+BC***
		Soil + ZnO NPs	Soil+ Biochar	Soil+ ZnO NPs+BC
	Treatments	0.4 g ZnO NPs	2% BC	0.4 g ZnO NP+ 2% BC
	Wt. of soil	350 to 400g /pot		
	Analyses	*pH, EC, Nutrient pool, O.M*		
		P+NP	***P+BC***	***P+NP+BC***
		Maize plant+NP	*Maize plant+Biochar*	*M.plant+ZnO NPs+Biochar*
	Treatments	0.7g ZnO NPs	2% BC	0.7 g ZnO NP+ 2% BC
	Wt. of soil	700 to 800g/pot		
	Analyses	*Proline,biomass,antioxidant enzymes(CAT,POD)*		

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3.3 Materials

To prepare the zinc oxide nanoparticles the main compound used is zinc acetate with a molar mass of 219.5g/mol. The sodium hydroxide (pellet.99%) was used to make the NaOH solution to optimize the pH of the mixture to basic. The production of zinc acetate was done through the biological method using *Corriandrum sativum* extract of leaves as a reducing agent. The distilled water is used as a solvent (Gnanasangeetha D & SaralaThambavani D, 2013). The pre-made *walnut-shell* biochar was used in contrast with ZnO NPs and a nano-biocomposite of biochar and ZnO NPs.

3.4 Preparation of treatments

3.4.1 Preparation of leave extract (*Corriandrum sativum*)

The *Corriandrum sativum* leaves were used to make the leaf extract. Fresh *Corriandrum sativum* leaves were collected and cut into shreds after that air dried at 50 °C for 30 minutes to remove any remaining moisture. The leaves were then repeatedly cleaned with water and deionized water to remove dust. The botanical extract was made by weighing 50 grams of dried and cleaned leaves in a 500-milliliter glass beaker and then adding 200 milliliters of deionized water. The mixture was brought to a boil until the aqueous solution turned a dark yellow. After cooling the extract to room temperature, Whatman filter paper (No.40) was used to filter it. The dark yellow to green color extract will be further used as a reducing agent (Ukidave & Ingale, 2022).

Figure 3.2 *Coriandrum sativum* leaves extract boiled and filtered.

3.4.2 Green synthesis of ZnO NPs

Green synthesis, known as biosynthesis, uses extracts from plants as the reducing agent along with microorganisms like yeast, fungus, algae, and bacteria (Akintlu & Folorunso, 2020). Mix 1 g of zinc acetate in distilled water (50 ml). The blend was stirred with a magnetic stirrer. 1ml of coriander leaf extract is added gradually while stirring. the concentrations of the zinc acetate are adjusted according to the required amount of the solution. The mixture was mixed for 2 hours continuously. Later the pH was checked with the help of a pH meter.

The NaOH 2M solution was added drop by drop to optimize the pH to basic until the combination pH of 12 was obtained. After this, the mixture was stirred for another 1 hour (Sabir et al., 2014). Once the mixture is stirred the solution is then centrifuged at 3000rpm for 40 minutes. After that, the separated nanoparticles were collected in the Petri dish discarding the separated liquid. The collected nanoparticles were oven-dried at around 60 to 80 C and dried nanoparticles were further crushed with pestle and mortar.

Figure 3.3 Preparation of ZnO NPs by Green-synthesis method.

3.4.3 Biochar

The biochar used as a treatment is walnut biochar. It's a pre-pared biochar the biochar preparation process involves collecting raw nutshell material, air-drying it, chopping it into pieces, and placing the sample in an oven at 105℃ for 1 hour to remove the remaining moisture. The dried material was pyrolyzed at 450℃ for 20 minutes, then cooled and stored in an airtight container with silica gel. To ensure uniform distribution, the biochar is sieved to a 0.5mm size and ground to a fine powder. (Qadeer et.al.,2014).

3.5 Characterization of synthesized NPS

Particles were characterized by doing SEM-EDS, FTIR, and EDX analysis Element and chemical information on the nanoparticles can be obtained using analysis that provides a general representation of the sample by determining its surface elemental composition and estimating their fraction at various positions (Anand Raj & Jayalakshmy, 2015).

3.5.1 Scanning Electron Spectroscopy

SEM analysis is a valuable technique for analyzing materials ranging from the nanoscale to the micrometer (μm) scale, including organic and inorganic materials. (Mohammed & Abdullah, n.d.).SEM/EDS for material analysis is a combination method that utilizes energy-dispersive X-ray spectroscopy and a scanning electron microscope. SEM does the imaging part, whereas EDS handles the detection. The scanning electron microscope employs electrons instead of light to produce an optical signal and EDS is applied for analysis and detection. EDS is a method for figuring out a material's chemical composition using electron microscopes (Newbury & Ritchie, 2013).

3.5.2 FTIR (Fourier transform infrared spectroscopy)

The spectral characteristics of amino acid sequences and cofactors are investigated using FTIR or Fourier transform infrared spectroscopy, which can measure even the smallest structural alterations. This method enables us to directly study the vibrational properties of nearly all cofactors, amino acid side chains, and water molecules. We can select vibrations related to specific chemical groups involved in a particular reaction using reaction-induced FTIR difference spectroscopy.

(Berthomieu & Hienerwadel, 2009). The term "Fourier transform infrared spectroscopy" refers to the scientific process of converting the two fields of frequency and distance into one another (Berna, 2017).

3.5.3 X-Ray Diffraction (XRD)

X-ray diffraction analysis (XRD) provides a non-destructive approach to acquiring extensive information on a material's chemical composition, physical properties, and crystallographic structure. (Garcia-Granda & Montejo-Bernardo, 2004).X-ray scattering (XRD) involves directing combined X-rays toward a nanomaterial sample. When the sample interacts with the incoming rays, a diffracted beam is formed, which is then detected, processed, and recorded. A diffraction pattern is created by plotting the intensity of the diffracted rays scattered at different angles of the material. This distinct X-ray diffraction pattern is to determine the crystal structure of the material by analyzing the reinforced XRD. (Chauhan,2014).

3.6 Soil

3.6.1 Soil sampling

Saline soil was collected from the agricultural land affected by salinity around Firoza, district Rahim Yar Khan. The sample was collected in zip bags and stored at a controlled temperature of 5C until required for experimental setup. Pre-existing saline soil was moderately saline soil S-1 and the other soil was collected from the normal land and made artificially saline ranging to highly saline soil S-2.

3.7 Pot experiment

Pot experimentation was done with saline soil to check the soil parameters and plant growth in the pots to determine the level of growth during the experiment. The soil was filtered by using a (5 mm) mesh sieve to remove rocks, dried matter, and all the unwanted materials to get the fine soil. The fine soil was measured and distributed among the pots respectively. The pot setup included both soils i.e. S-1 (Moderately saline) and S-2 (Highly saline soil).

Figure 3.4 S-1 and S-2 placement in pots.

3.8 Chemical parameters of soil

Chemical analysis of the soil was conducted using the protocols mentioned and the use of a multimeter to check the parameters such as pH, and EC.

3.8.1 pH

The 5 g of soil sample was collected from the pot. The soil was combined thoroughly with 50ml of distilled water. The sample was stirred gradually until mixed and then filtered. The sample was tested with a multimeter to check the pH of the respective samples.

3.8.2 Electrical Conductivity

The prepared soil samples were used to check the EC as well with the multimeter.

3.8.3 Organic matter

The method that was followed to determine the O.M was a loss on ignition direct estimation of soil's organic matter. The O.M. was measured by taking 10g of the soil sample. The soil sample was oven-dried at 100 C for 1 hour to remove the moisture content and measured again to check the weight. Weight loss is considered as moisture content removal from the soil. The sample was heated in the furnace at 400 C. The sample was kept in the furnace for 2 hours to remove the organic content of the soil. Weight loss after the ignition is considered the organic matter content (Robertson, 2011).

$$Organic\ matter\% = \frac{(W_3 - W_2)}{(W_2 - W_1)} \times 100$$

3.9 Nutrient pool analysis

3.9.1 Nitrogen

The nitrogen present in the soil was measured by nitrogen by nitrate method. The 5 g of soil was measured and mixed with distilled water (50 ml). The prepared sample was measured with a UV spectrophotometer at 220nm. The values were further calculated using the formula (Allende-Montalbán et al., 2024). The values were further evaluated using the formula given.

$$Conc.\ of\ sample = \frac{ABS.of\ sample \times Conc.of\ standard.}{Abs.of\ standard}$$

3.9.2 Potassium

The potassium in the soil was measured by 5 g of mixed soil with 50 ml of distilled water. The water extract can determine the soluble K (Estefan et al., 2013) The prepared sample's absorbance was measured at 766 nm. The obtained absorbance was calculated according to the curve made of the potassium in the soil.

3.9.3 Phosphorus

An essential apparatus used in the laboratory procedure for phosphate determination was a spectrophotometer set to a specific wavelength of 882 nm to calculate the phosphorus content in the soil. Re agents included 5 N NaOH prepared by dissolving 200 g NaOH in distilled water; 0.5 M NaHCO3 adjusted to pH 8.5 with 5 N NaOH and 5 N H2SO4 diluted from concentrated acid. Special reagents included p-nitrophenol indicator, Reagent-A (ammonium heptamolybdate and antimony

potassium tartrate in sulfuric acid), and Reagent-B (L-Ascorbic acid mixed with Reagent-A).

A Standard Stock Solution was prepared from dried KH2PO4, providing 500 ppm P, and diluted to create standard solutions with known concentrations of P (1, 2, 3, 4, and 5 ppm P). These components are utilized sequentially to measure phosphate levels via UV spectrophotometer analysis (Niaz et al.,2024). The absorbance was checked at 882 nm wavelength in UV-spectrophotometry. The values were further calculated by using a given formula as per protocol for extractable phosphorus.

$$Extractable\ P(ppm) = ppm\ P \times \frac{V}{Wt} \times \frac{V_2}{V1}$$

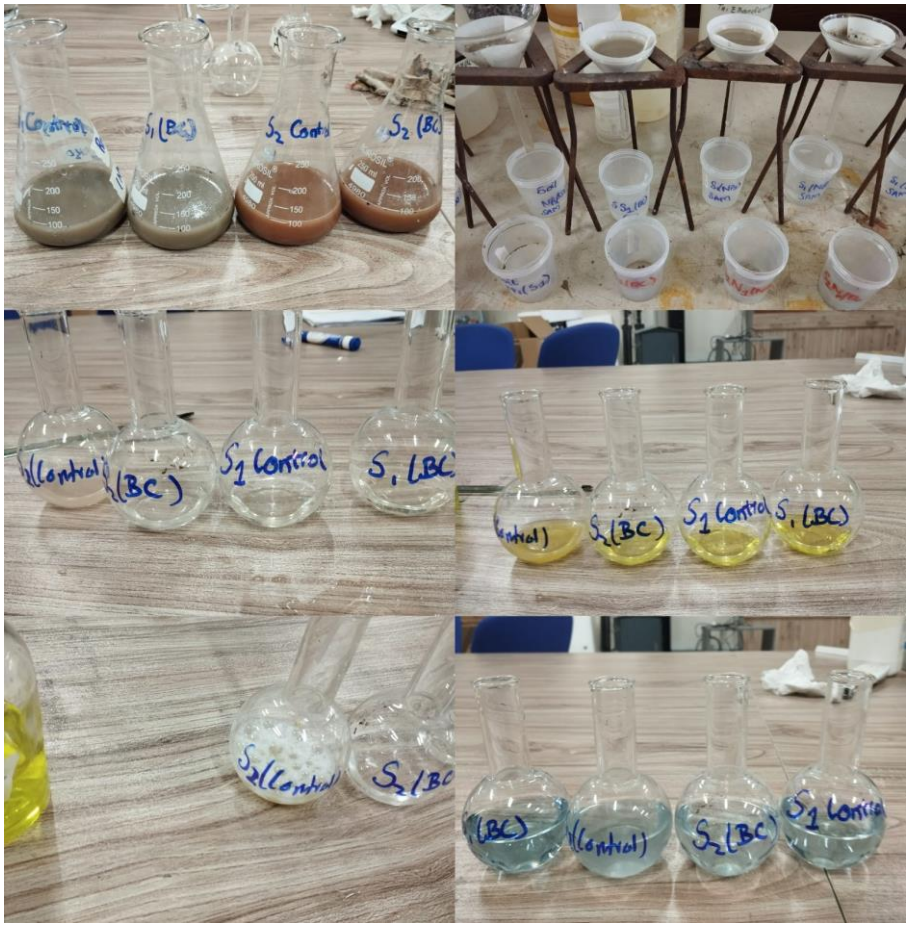


Figure 3.5 Sample preparation for phosphorus in soil.

3.10 Plant

Plant was used to determine the growth rate and effect on it during a stress-induced environment. For these maize seeds were used keeping weather and growing time in mind. The changes in the plants after being induced by stress conditions were noted by doing different plant analyses.

3.10.1 Maize seeds

Maize seeds were washed with distilled water and dipped in ethanol mixed with a distilled water solution for surface sterilization. The seeds were taken out of the solution. The filter paper was placed on the petri dish, seeds were placed vertically in 2 rows containing 3 seeds each. Seeds were sprayed with distilled water to make it damp. Another filter paper was placed on the seeds to cover them, and the lid of the petri dish was sealed from the side with the help of tape. To avoid any fungal growth or cross-contamination. The seeds were then incubated for 48 hours or until the roots developed.

Figure 3.5 Sterilization, incubation and planting of seeds.

3.10.2 Plantation

The sprouted seeds were then planted in the normal soil given favorable conditions to grow. Until the growth of 7-10 days was achieved and leaves developed. The plants were extracted carefully so as not to disrupt the roots, and they were planted into the saline soil and induced with treatments.

3.11 Plant analysis

The plant's enzyme activities were monitored to test stress response. The plants were tested to evaluate their growth and response to stress through proline and antioxidant estimation.

3.11.1 Proline

Plants under stress, especially osmotic stressors, commonly exhibit overproduction of proline as a response. Determining this amino acid helps evaluate physiological status and, in general, comprehend plants' ability to withstand stress. We present a straightforward, quick, and generally safe ninhydrin-based technique (Carillo & Gibon, 2011). Proline is examined from this method using fresh leaf tissue. To precipitate proteins, 250–500 mg of tissue was first ground up using a cold pestle and mortar then homogenized 5 mL of 3% sulphosalicylic acid. Whatman Filter paper No.2 was used to filter the homogenate that was produced. To create a colored complex, 2 mL of the filtered sample is then combined with 2 mL of acid-ninhydrin along with glacial acetic acid in test tubes. The mixture is then agitated and incubated at 100 °C for an hour with a water bath. The test tubes were then chilled in an ice bath. Each tube receives 4 mL of toluene, which is then vortexed to separate the

aqueous and organic phases to extract the pink layer formed. The pinkish mixture was then collected by using a pipette. Measured is the absorbance of the prepared sample at 520 nm in a UV spectrophotometer (Tamayo & Bonjoch, 2006). The values were further evaluated by using the given formula.

$$Proline = \frac{Abs \times Total\ sample}{Molecular\ weight}$$

Figure 3.6 Sample preparation for proline testing.

3.11.2 Biomass

The total weight of living plant material in a specific area or volume is referred to as plant biomass. It is usually expressed as dry biomass or fresh weight (wet biomass). Measurements of biomass serve as important indicators of the health, growth, and productivity of plants. Plant biomass was measured by extracting the plant from the soil and measuring it on the measuring scale. The entire plant was

measured, and the value was noted in grams. After that the plants were oven-dried for 72h and again the plants were measured. Both the values were subtracted. The weight difference was computed as the weight of the biomass (Roberts et al., 1985).

3.11.3 Enzymatic Antioxidant Estimation

3.11.3.1 Catalase

Firstly, the sample was prepared. The leaves were crushed with the 0.1 M phosphate buffer in the cold pestle and mortar. The extract was then collected in Eppendorf tubes. The sample was then centrifuged at 1200 rpm for 24 minutes. After that, the dense particles were settled in the bottom of the Eppendorf whereas the liquid layer was collected. In the collected sample hydrogen peroxide and phosphate buffer were added to get the volume of 3ml (Sarker & Oba, 2018). The absorbance was checked at 240nm in a UV spectrophotometer. Catalase values were further calculated by using the formula given with 39.4 $Mm^{-1}cm^{-1}$ as the coefficient as per the protocol.

$$Catalase\ activity = \frac{(A_1 - A_2) \times V \times DF}{T \times \varepsilon}$$

3.11.3.2 Peroxidase (POD)

The sample was prepared by taking the maize plant leaves to analyze the peroxidase enzyme in the plant. The leaves were crushed through pestle and mortar by adding phosphate buffer until pH 7 was achieved. The prepared sample was then

centrifuged at 2400 rpm for 15 minutes. The separated liquid sample was then collected. The 0.1 substrate, which was 4-methyl pyrocatechol added to 1 ml of plant extract. 2 ml of phosphate buffer and 2 ml of hydrogen peroxide were added to the sample (Senthilkumar et al., 2021). The sample absorbance was checked at 420 nm in a UV spectrophotometer. Peroxidase values were further calculated by using the formula given with 2.8 $Mm^{-1}cm^{-1}$ as the coefficient as per the protocol.

$$POD = \frac{Abs \times Total\ volume}{\varepsilon \times Enzyme\ volume}$$

Figure 3.7(a) Sampling preparation for CAT and POD.

Figure 3.7(b) Prepared sample of CAT and POD.

3.12 Zinc analysis

For the analysis of Zinc in soil digestion of soil was done. About 10 g of soil was taken and air dried. After that, the soil was mixed with the perchloric acid $HCLO_3$. The mixture was stirred after a few minutes several times. The sample was heated on the heating plate for around 20 minutes at 100C. After that 30ml of D.W was added. The sample was later filtered using Whatman filter paper. The samples were sent for analysis from the ICP-oes instrument.

Figure 3.8 Soil digestion to prepare the sample for Zinc analysis.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter examines the results of the NP and BC treatment for the removal of salinity stress in the soil. It also includes the removal efficiency of zinc oxide nanoparticles. The zinc nanoparticles and walnut biochar were used to treat soil to check their effectiveness in alleviating salt stress from the soil. The synthesized nanoparticles were characterized through FTIR, SEM-EDS, and XRD. The results include the efficacy of nanoparticles and their effects on the soil nutrient pool. Plants' antioxidants and proline were also tested to check the impact of stress levels on the plants.

4.1 Characterization of zinc oxide nanoparticles

4.1.1 Scanning electron Microscopy SEM-EDS

The scanning electron microscopy (SEM) image was taken at a magnification of 3000x, displaying the surface morphology of the zinc oxide nanoparticles (ZnO NPs). The scale bar of 5 µm indicates that the observed clusters or agglomerates are nano. The surface indicates the presence of mesopores, which are commonly

characterized as pores with widths ranging from 2 to 50 nm. Mesoporous ZnO has been demonstrated to have greater reactivity because of its increased surface area, which increases its interaction with target molecules (Uribe-López et al., 2021).

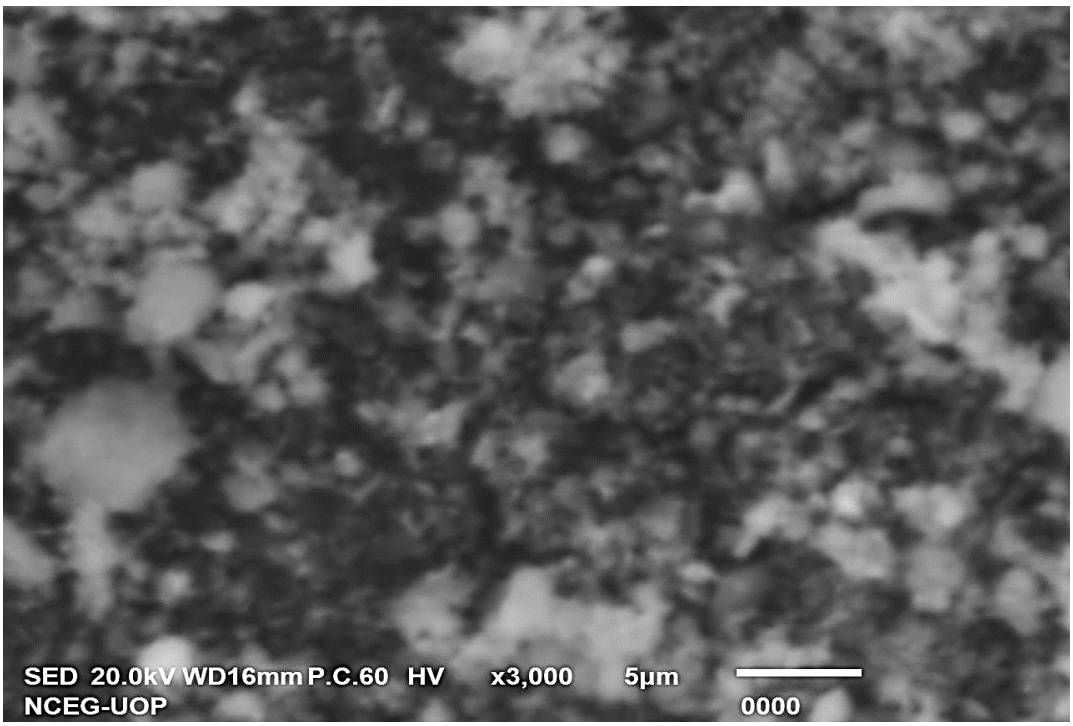
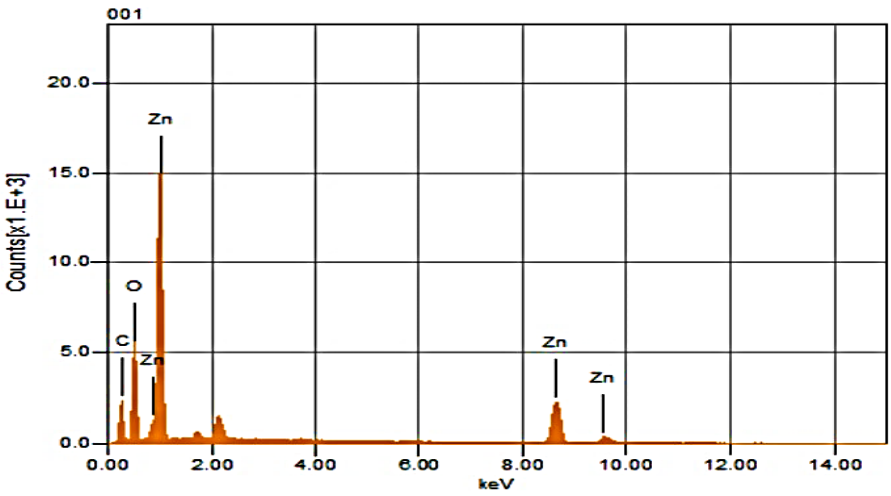


Figure 4.9 SEM revealed that ZnO NPs are mesoporous

This morphology's high surface area improves the functional characteristics of ZnO NPs, making them suitable for beneficial and adsorptive applications (Gaur et al., 2024).The results reveal that when ZnO NPs are generated via processes like sol-gel or precipitation, they frequently exhibit similar morphological traits, with aggregated structures and high surface areas being typical (Raha & Ahmaruzzaman, 2022).

The EDS spectrum verifies the expected composition of the ZnO nanoparticles, with high levels of zinc and oxygen suggesting the existence of zinc oxide. The zinc peaks in the spectrum confirm the crystalline nature of ZnO, which usually has a wurtzite structure. The EDS analysis shows a 40% mass of zinc,34% of oxygen, and 24% of carbon. The SEM and EDS result outcome shows the effective production of ZnO

nanoparticles. The basic composition, which consists mostly of zinc and oxygen, supports the presence of ZnO. The EDS examination yielded the following elemental composition for the ZnO nanoparticles as shown in graph details.



Formula	mass%	Atom%	Sigma	Net	K ratio	Line
C	24.53	42.26	0.02	58762	0.0074118	K
O	34.64	44.81	0.06	147842	0.0852154	K
Zn	40.83	12.93	0.13	182410	0.2128688	K
Total	100.00	100.00				

Figure 4.10 EDS analysis shows a 40% mass of zinc,34% oxygen, and 24% carbon

4.1.2 Fourier transforms infrared spectroscopy FTIR.

ZnO nanoparticle purity and crystallinity were evaluated using FTIR spectroscopy, and these factors impact the nanoparticles' optical and electrical characteristics. Identifying distinct vibrational modes linked to Zn-O FTIR data, the major peaks and the wavenumbers that correspond to them were examined. The peaks show the existence of functional groups and absorbance identification. The broad absorption band around 3421.83cm-1 indicates O-H stretching vibration and the presence of hydroxyl groups on the surface of the ZnO nanoparticles. The absorption peak at 2920.32 cm-1 and 2850.68 cm-1 shows the C-H stretching vibrations

The peak at 1744.08 cm-1 indicates the C=O stretching associated with carbonyl groups, which helps stabilize the ZnO nanoparticles. The peak at 1637.62

cm-1 represents the presence of absorbed water on the surface of the ZnO nanoparticles. The peaks at 1411.94 cm-1 and 1508 cm-1 are attributed to the stretching vibrations of C-O bonds. The sharp peaks at 914.29cm-1 and 759.98cm-1 observed indicate that the Zn-O bonds are present. The sharp peaks are characteristic of ZnO, confirming the formation of ZnO nanoparticles. The peaks at 689.32 and 430.14 cm-1 also confirm the presence of Zn-O bonds which confirms the formation of ZnO nanoparticles.

The FTIR spectra have several important characteristics that attest to the synthesis's success and the existence of functional groups is shown by the significant Zn-O stretching vibrations at 914.29 cm^{-1}, 759.98 cm^{-1}, 669.32 cm^{-1}, and 430.14 cm^{-1}. The large absorption band at 3421.83 cm^{-1}, indicates the presence of hydroxyl groups implying ZnO nanoparticles have hydroxyl groups bonded to their surface. Contributing to their stability and dispersibility in aqueous or other materials (Gultepe et al., 2023).

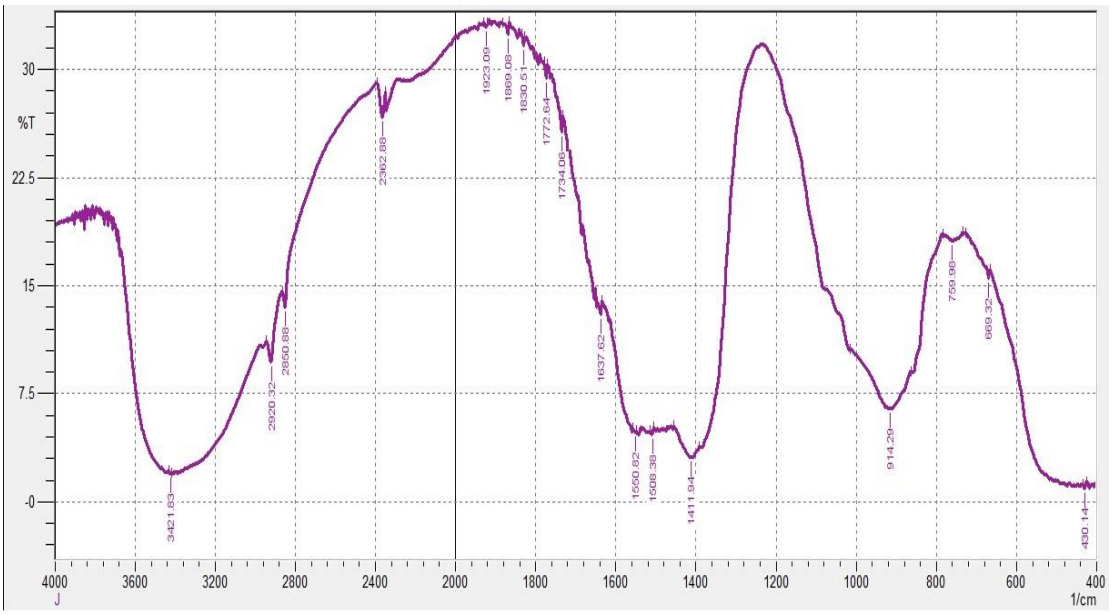


Figure 4.11 Peaks at 430 and 669cm^{-1} confirm the presence of ZnO NPs.

The effective production of ZnO nanoparticles with distinctive Zn-O bond vibrations is confirmed by the FTIR analysis. The spectrum also indicates the presence of organic residues, absorbed water molecules, and surface hydroxyl groups. These functional groups may have an impact on the solubility, stability, and

interaction of the nanoparticles with other chemicals in biological systems, sensing, or catalysis, among other applications (M. F. Khan et al., 2016).The results are in line with the expected chemical structure and composition of the particles (I. Khan et al., 2019).

Table 1.4.1.2 FTIR functional groups

Absorbance (cm^{-1})	**Functional Group**
3421.83	Hydroxyl (–OH)
2850.68	Aliphatic C-H ($–CH_2$, $–CH_3$)
1744.08	Carbonyl (C=O)
1637.62	Water (H_2O)
1508.36	Carboxylate (COO^-)
669.32	Zinc Oxide (Zn-O)
430.14	Zinc Oxide (Zn-O)

4.1.3 X-Ray Diffraction XRD

The diffraction pattern of zinc oxide (ZnO) nanoparticles exhibits clear peaks at specific 2θ values between 30° and 100°, corresponding to different crystal planes and indicating the hexagonal wurtzite structure of ZnO. Specifically, the prominent peaks are observed at approximately 31.7°, 34.4°, 36.3°, 47.6°, 56.7°, and 62.8°,

corresponding to the (100), (002), (101), (102), (110), and (103) planes of ZnO, indicate the crystalline nature and good crystallinity in the ZnO nanoparticles. The wurtzite structure is typical for ZnO, due to its thermodynamic stability under ambient conditions (Shaba et al., 2021).

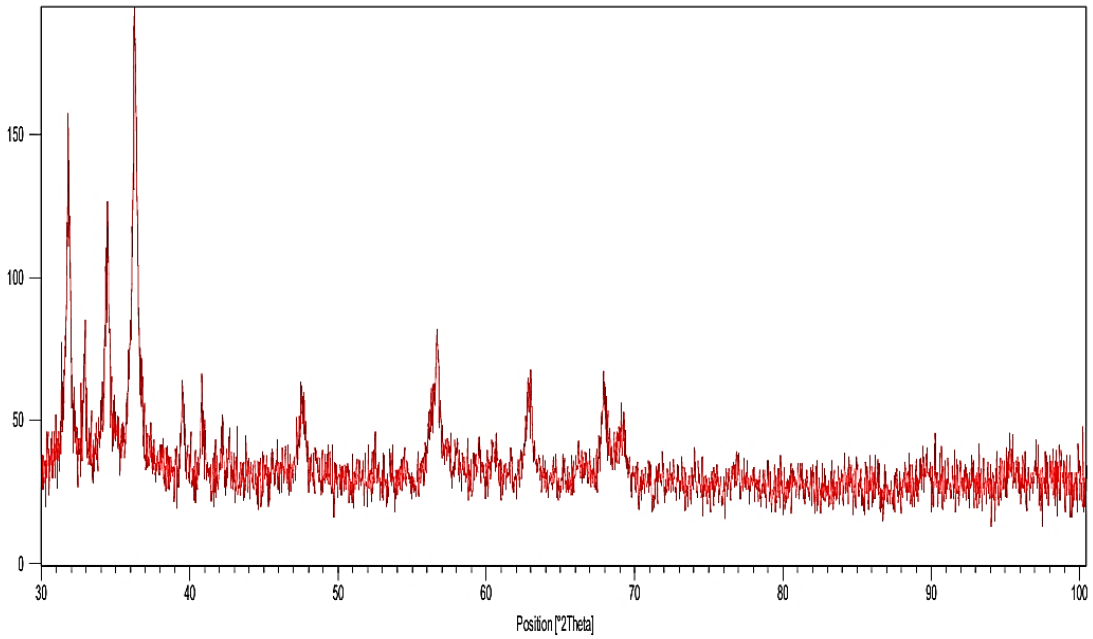


Figure 4.12 XRD prominent peaks represent the crystallinity of ZnO

4.3 Soil analysis

The soil was analyzed to determine the effect of treatments on the soil nutrient pool and determine changes in pH and electrical conductivity. The chemical analysis included pH and EC. The nutrient pool was also analyzed to determine the changes in nutrient values.

4.3.1 Chemical analysis

4.3.1.1 pH

The control treatment entirely represented stress conditions without amendments. In control S-1 pH level was 8.83 to 8.81. The S-2 pH level was initially recorded at 8.74 to 8.65 in the final reading. Both controls showed an inadequate decrease in the pH level indicating that the soil itself is not suppressing salinity. Salt buildup in the soil alters its composition, deteriorates its properties, and raises its pH. Although the pH (>8.5) of saline soil is high, an excess of soluble salt in the subsoil limits crops' ability to absorb water (Akhtar, 2019).

In S+NP, ZnO NPs were incorporated in the S1 and S2. Initial pH values were 8.61 and 9.67, respectively. After 15 days of treatment, the plant pH values dropped to 7.58 and 7.25, respectively, demonstrating NPs' strong ability to neutralize salinity, especially in the high saline condition S2. In P+NP, S1 pH showed a decrease in salinity from 8.95 to 7.82, whereas S2 pH levels were reduced from 8.81 to 7. 49, highlighting the consistent impact of NPs in reducing salinity levels. +BC treatment also resulted in pH reductions from 8.56 to 7.28 in S1 and 8.94 to 7.21 in S2, indicating BC's effectiveness in improving the soil conditions.

In P+BC biochar led to reductions from 8.71 to 7.58 in both soil types demonstrating its broad applicability in pH moderation. S+NP+BC showed a balanced reduction in pH from 8.81 to 7.98 in S1 whereas in S1 it reduced from 9.11 to 7.41, indicating synergistic effects of NP+BC in pH stabilization. P+NP+BC treatment reduced Ph from 8.56 to 7.68 in S1 and from 8.55 to 7.53 in S2, showing a consistent reduction but less pronounced than NP or BC alone.

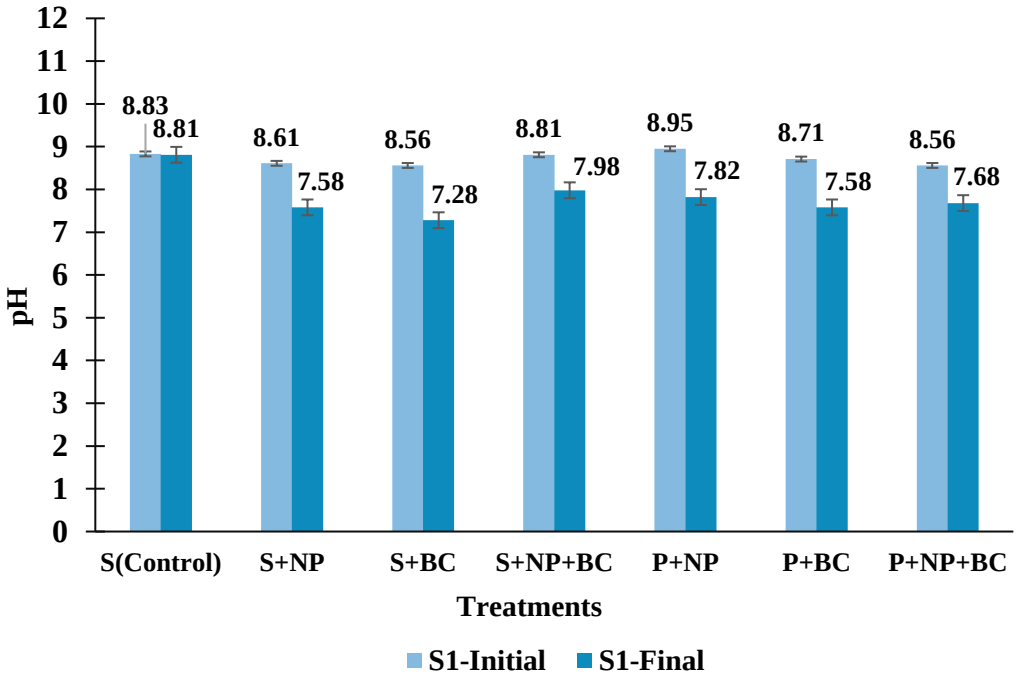


Figure 4.13(a) Result of pH for S-1

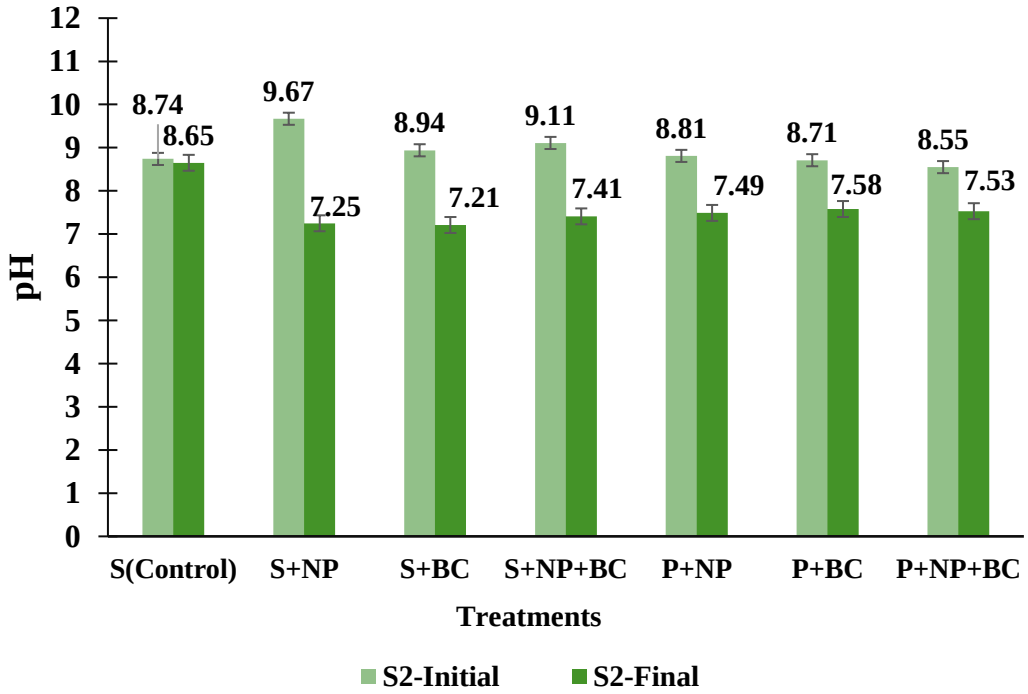


Figure 4.13(b) Result of pH for S-2

4.3.1.2 Electrical conductivity

Electrical conductivity in the S (Control), EC increased slightly in both S1 from 4.52 to 4.6 and S2 from 10.07 to 10.5 indicating that without any treatment the salinity levels tend to rise naturally due to the accumulation of salts (Maciej Serda et al., 2013). The S+NP treatment decreased EC, particularly in S1 from 3.94 to 3.45 indicating that ZnO NPs reduced salinity to some extent. Similarly, in S2 the value reduced from 11.22 to 8.78 suggesting that ZnO is efficient in alleviating salt stress. In both soil types, the P+NP treatment resulted in a significant decrease in EC from 5.08 to 4.15 and 10.95 to 8.92 in S1, and S2 respectively. This suggests that the ZnO NPs improve salt leaching and strengthen plant capacity to tolerate salinity (Basit et al., 2022).

Similarly, the S+BC treatment in S1 was reduced from 3.7 to 3.45 which indicates a slight reduction by the application of biochar. It also showed a reduction in EC, in S2 from 11.3 to 9.57, which is assigned to biochar's ability to adsorb salts and improve soil structure, thus mitigating salinity. In S1, P+BC reduced from 4.52 to 4.35., particularly in S2 from 10.01 to 9.37 This might be a result of the high porosity and ability of biochar with a high carbon-to-nitrogen content to absorb more salt, thereby lowering the salinity of the soil (Wu et al., 2024).

The S+NP+BC in S1 values reduced from 4.16 to 3.3. S2 had the most stated effect, leading to a substantial decrease in EC in S2 from 11.55 to 8.65. in the S1 combined treatment, EC reduced from 4.59 to 4.22 and the P+NP+BC treatment in S2 resulted in a significant decrease in EC, especially in S2 from 9.55 to 8.08, reflecting the combined treatment is efficient in reducing salinity (Ahmad et al., 2024).

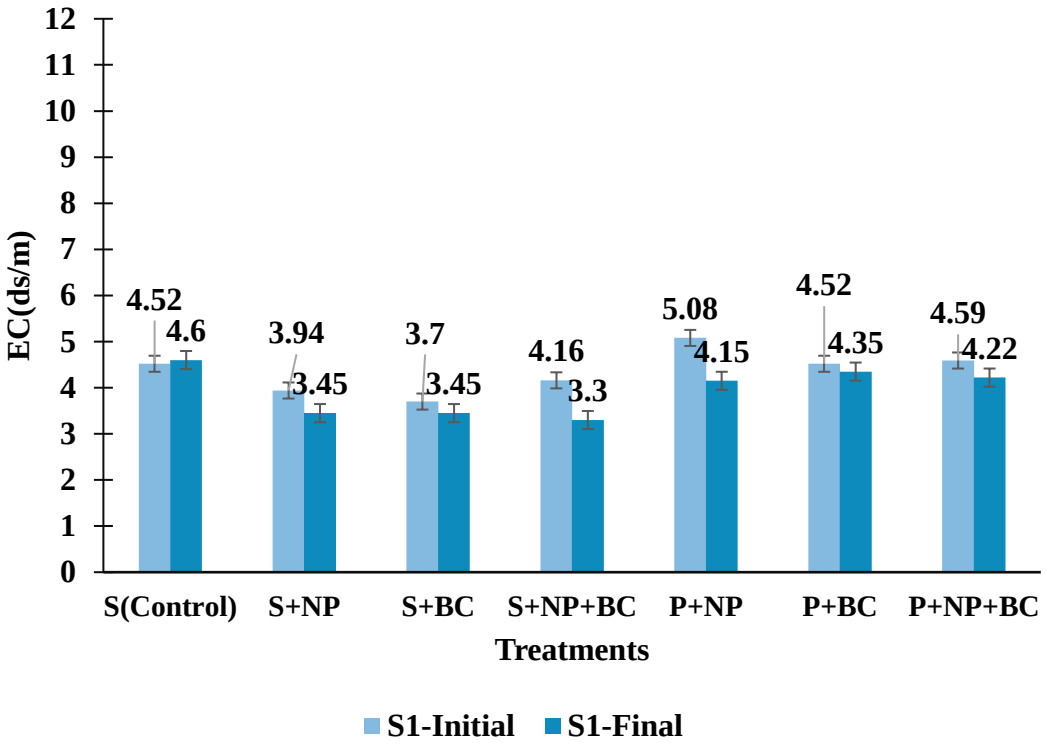


Figure 4. 14(a) Results of EC(ds/m) for S-1

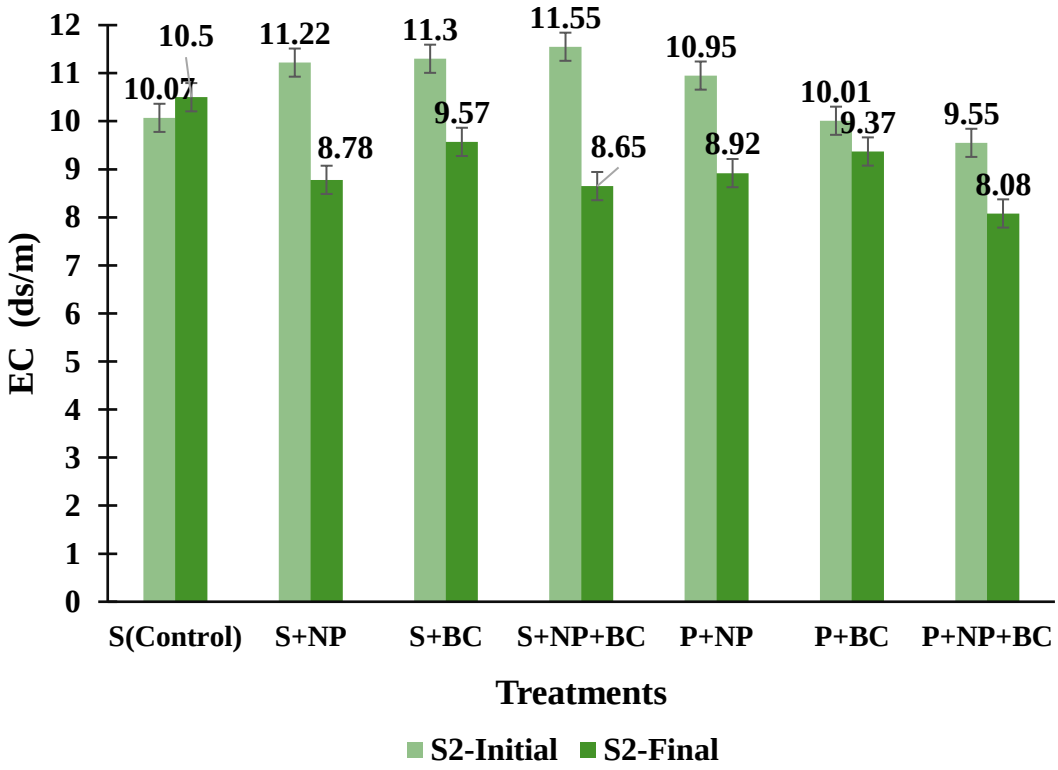


Figure 4.14(b) Results of EC(ds/m) for S-2

4.3.2 Nutrient analysis

4.3.2.1 Nitrogen

The nitrogen (N) content results indicate the influence of different treatments on S1 and S2. In the control group (S), nitrogen levels increased in S1 from 10.02 to 13.32 mg/kg and S2 from 3.33 to 6.4 mg/kg, suggesting a natural rise in nitrogen levels over time without any treatments. Applying the S+NP treatment led to a substantial increase in nitrogen levels. In the S1 soil, nitrogen content rose from 9.52 to 28.83 mg/kg, and in the highly saline S2 soil, it increased from 2.61 to 23.32 mg/kg. This underscores the role of zinc nanoparticles in increasing nitrogen uptake by improving root function and nutrient availability in saline conditions. In contrast, the P+NP treatment exhibited the highest increase in nitrogen levels, particularly in S1 soils, where it increased from 11.83 to 64.66 mg/kg. In the S2 soil, nitrogen levels increased from 1.13 to 18.46 mg/kg (Alabdallah & Alzahrani, 2020).

Under the S+BC treatment, nitrogen levels increased significantly in S1 soils from 8.95 to 23.34 mg/kg, and in S2 from 2.93 to 23.06 mg/kg. In the P+BC treatment, nitrogen levels increased in both S1 soils from 9.523 to 25.37 mg/kg and S2 from 2.439 to 12.88 mg/kg, indicating that biochar helps stabilize nitrogen levels in saline soils. When assessed to the control, the soil nitrogen level increased by 16.35% after applying Biochar (Zaheer et al., 2021).

Applying S+NP+BC treatment significantly increased nitrogen content in S1 from 10.4 to 26.8, especially in S2, from 2.36 to 24.19 mg/kg. This demonstrates the combined effect of BC and NPs in boosting nitrogen retention and mitigating the bad effects of salinity. Furthermore, the P+NP+BC treatment led to substantial increases in nitrogen in S1 soils, from 10.85 to 57.65 mg/kg, but showed a minor increase in S2, from 1.73 to 10.9 mg/kg. This indicates that while the combination effectively improves nitrogen retention in S1 soils, it encounters challenges under extreme salinity.

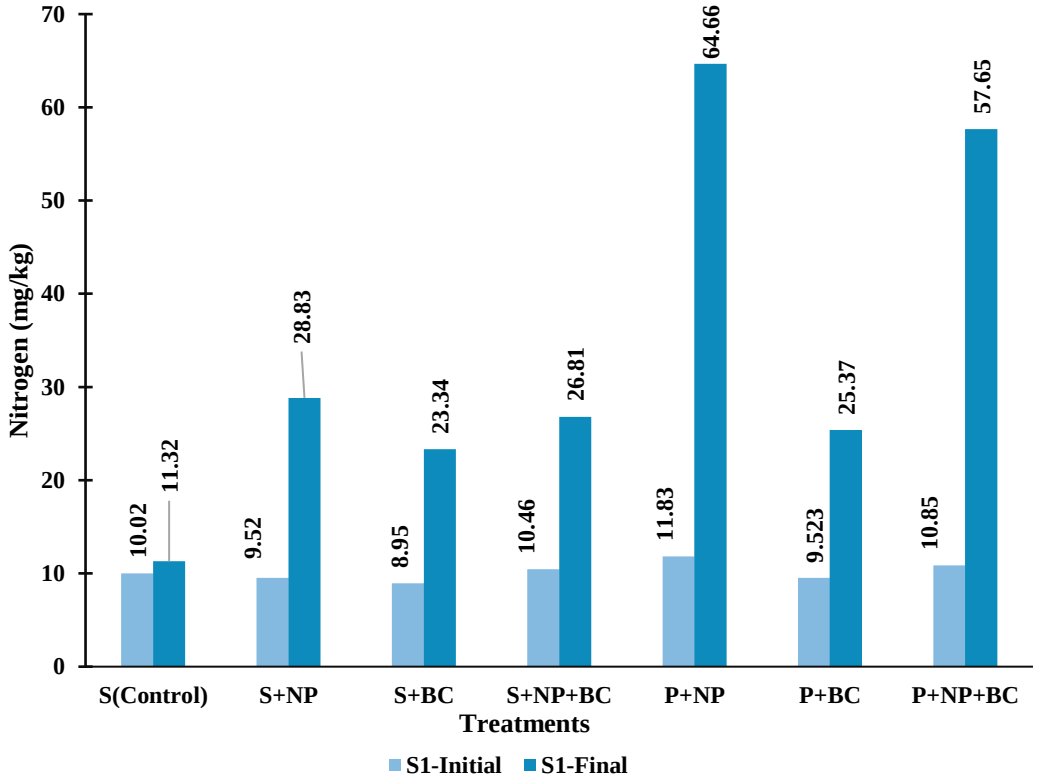


Figure 4.15 (a) Results of Nitrogen in S-1

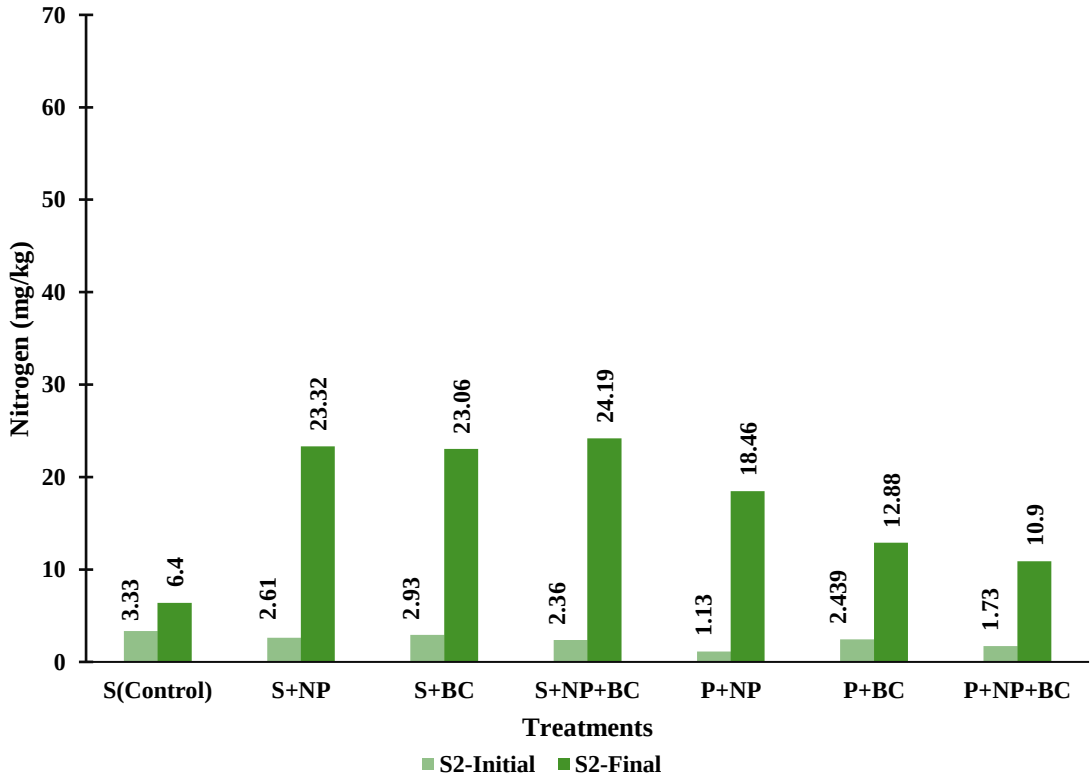


Figure 4.15 (b) Results of Nitrogen in S-2.

4.3.2.2 Potassium

In the control group (S), potassium levels in S1 marginally decreased from 0.002 to 0.001 mg/kg, while in S2, t from 0.007 to 0.005 mg/kg. The decrease in response to stabilizing nutrients of soil in salinity. In the case of the S+NP treatment, potassium levels exhibited a significant increase in both S1 from 0.001 to 0.008 mg/kg and in S2 from 0.025 to 0.109 mg/kg. Similarly, the P+NP treatment led to a notable increase in potassium in S1 soil from 0.005 to 0.042 mg/kg, while in S2 soil from 0.002 to 0.008 mg/kg. The enhancement of potassium retention in S1 soil is likely attributed to the nanoparticles' ability to improve root function and nutrient absorption under stress.

Under the S+BC treatment, potassium levels increased in both soil types, particularly in S1 from 0.009 to 0.069 mg/kg. This could be ascribed to the capability of biochar to stabilize potassium ions. Conversely, for the P+BC treatment, potassium levels remained relatively stable in S1 from 0.001 to 0.002 mg/kg but increased in S2 from 0.004 to 0.024 mg/kg. The adding of biochar to salt-affected soils improves the uptake of potassium (K^+) and decreases the uptake of Na^+, thus enhancing plant performance under salinity stress(Wu et al., 2023).

The combined S+NP+BC treatment demonstrated an increase in potassium levels in S1 from 0.014 to 0.033 mg/kg and in S2 from 0.002 to 0.025 mg/kg. The synergistic effects of biochar and zinc nanoparticles have contributed to improved nutrient retention and prevented potassium losses under salinity stress. Lastly, the P+NP+BC treatment resulted in an increase in potassium in S1 from 0.006 to 0.008 mg/kg and in S2 from 0.015 to 0.025 mg/kg. The combination of phosphorus, nanoparticles, and biochar may have increased or reduced potassium in soil.

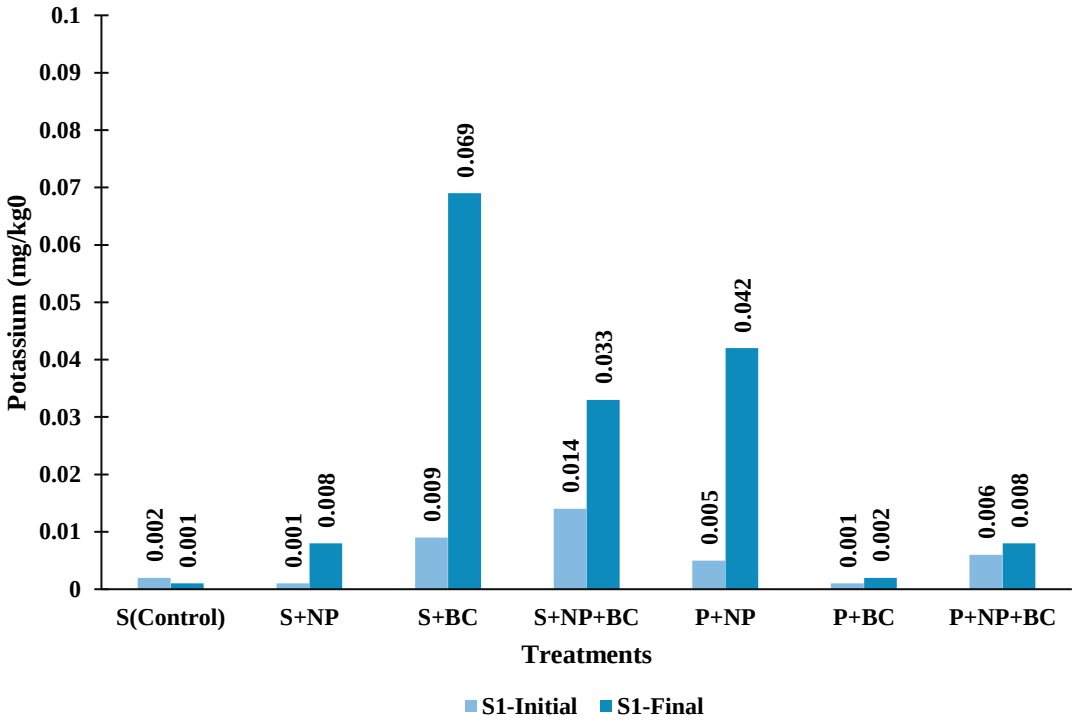


Figure 4.16 (a) Results of potassium in S-1.

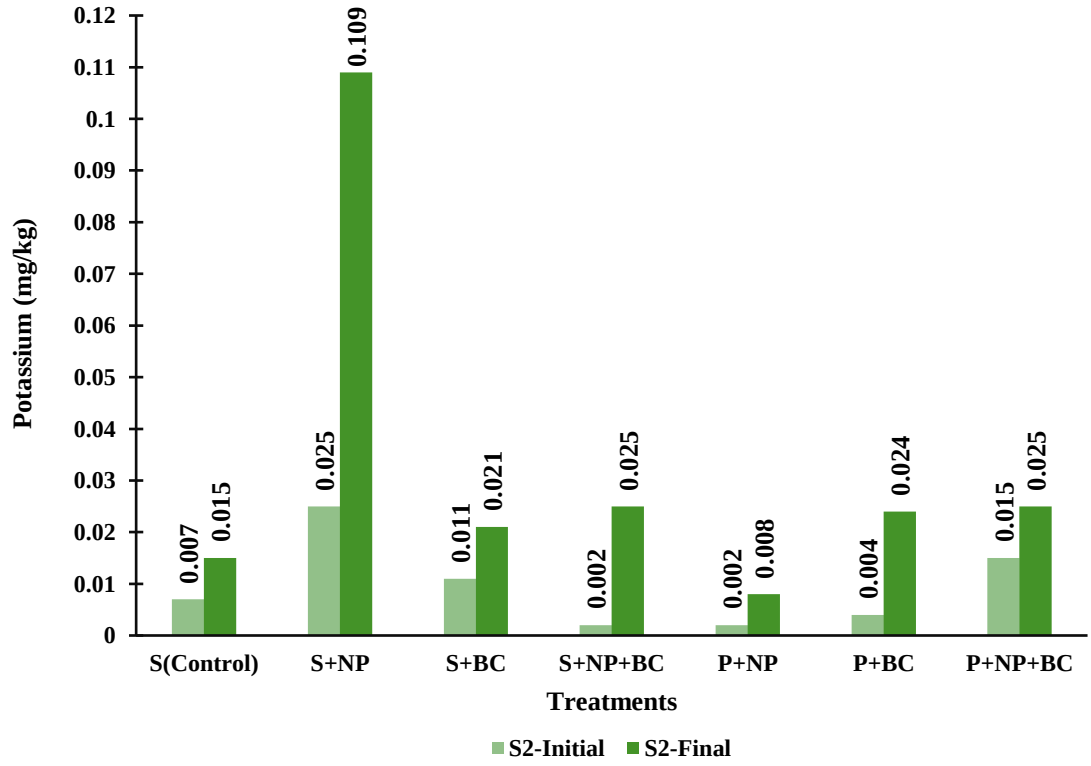


Figure 4.16(b) Results of potassium in S-2

4.3.2.3 Phosphorus

The analysis of phosphorus (P) levels demonstrates the distinct impact of each treatment on S1 and S2. In the control in S1 soil from 8.2 to 9.1 mg/kg, a slight decrease was observed in S2 from 23 to 21.2 mg/kg. This could be the natural fluctuations compared to the treated values evident increase was seen in treated soils. Consequently, in saline conditions characterized by high pH, high conductivity, and low soil organic matter, phosphorus availability was reduced, exacerbating phosphorus deficiency in plants (Xie et al., 2022).

The S+NP treatment exhibited an increase in phosphorus in both soil types. Specifically, phosphorus levels increased from 10.5 to 17.2 mg/kg in S1 and from 13.2 to 18 mg/kg in S2. This is associated with the potential of zinc nanoparticles to enhance phosphorus uptake by plants through improved root function and enzyme activity, thus mitigating the negative impacts of salinity stress. Conversely, the P+NP treatment increased phosphorus levels in both types of soil, with a more pronounced increase in S1 soil from 17 to 32.8 mg/kg compared to S2 from 12.8 to 18.6 mg/kg. Among the three soil types - non-salinized, slightly salinized, and moderately salinized soil - the phosphorus availability was the lowest in moderately salinized soil (MSS) with a salt content of 2.53 g kg^{-1}. The highest level of available phosphorus was recorded in the control non-saline soil at 16.17 kg ha^{-1}(Bala et al., 2019).

The S+BC treatment led to a substantial decrease in phosphorus levels in S1 from 53.3 to 19.7 mg/kg and in S2 from 13.1 to 10.3 mg/kg. This decline is presumably due to biochar's robust adsorption properties, which can immobilize phosphorus in the soil, thereby reducing its availability to plants under saline conditions. Similarly, the P+BC treatment increased phosphorus levels in S1 from 13.7 to 15.5 mg/kg and in S2 from 6.1 to 16.1 mg/kg. Soil salinity increased, and phosphorus availability decreased significantly, suggesting that soil salinization may inhibit soil phosphorus availability. Additionally, a negative connection between salt matter and available phosphorus was observed (Xie et al., 2022).

The S+NP+BC yielded stable phosphorus levels in S1 from 11.9 to 15.7 mg/kg, an increase was observed in S2 from 15.6 to 67.6 mg/kg. This increase in S2 is attributable to the combined action of NP+BC which improved phosphorus retention and subsequent availability to plants, particularly in extreme salinity conditions. Lastly, the P+NP+BC treatment led to minor increases in S1 from 15.5 to 17.6 mg/kg and increased phosphorus levels in S2 from 14.4 to 24.8 mg/kg. The combined effects of P+NP+BC contributed to stabilizing phosphorus in highly saline environments. The results indicated that the available phosphorus in non-salinized soil was drastically higher than that in salinized soil (Bala et al., 2019).

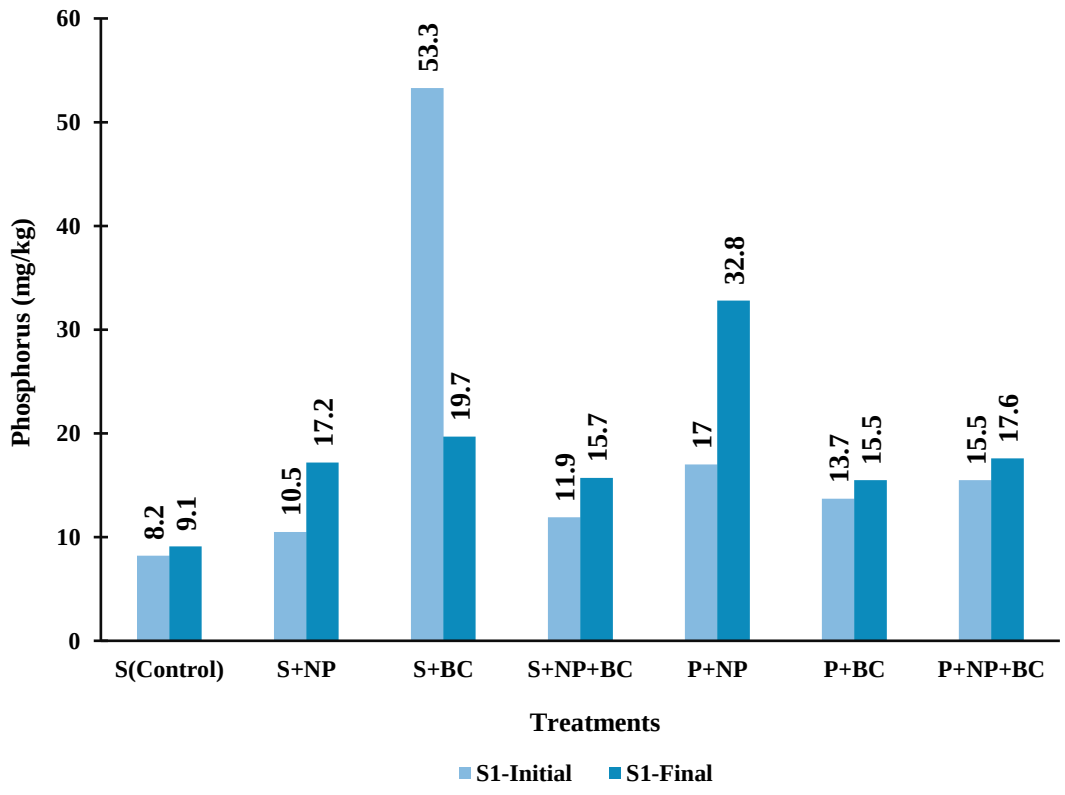


Figure 4.17(a) Results of phosphorus in S-1

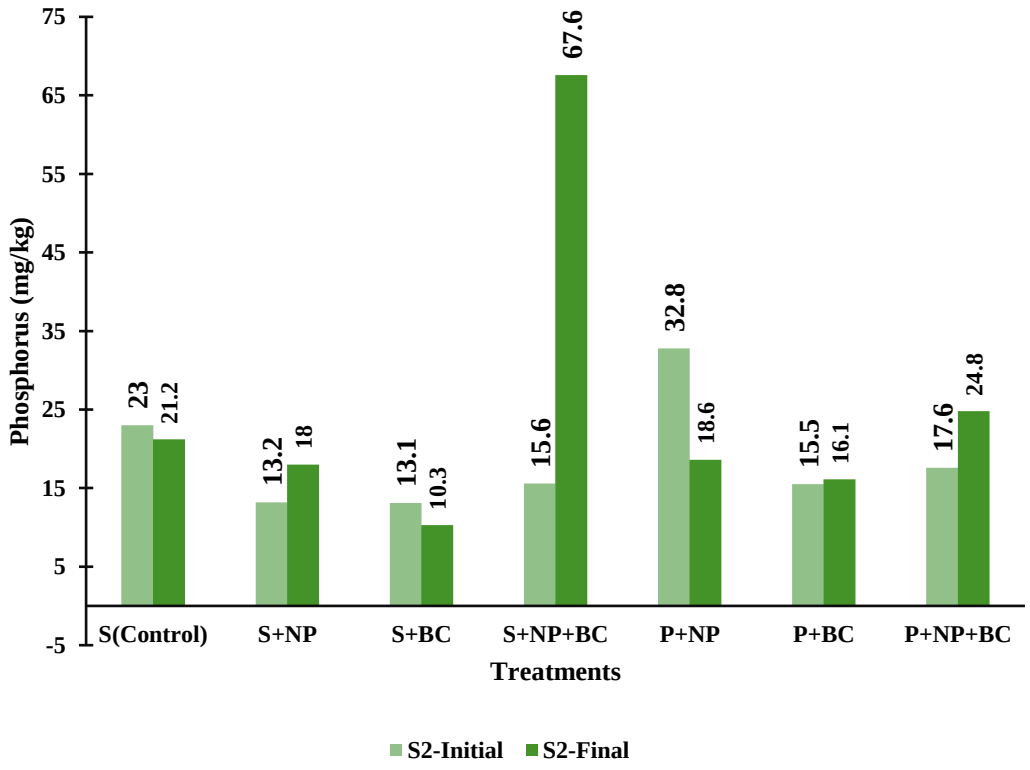


Figure 4.17(b) Results of phosphorous in S-2.

4.3.2.4 Organic matter

The analysis of organic matter in saline soil treated under various conditions consistently demonstrates a significant increase in organic matter content across most treatments, both in moderate and highly saline conditions. In the S(Control) group, there was a slight reduction in organic matter content, with levels r from 60 to 53 in S-1 and from 51 to 46 in S2. (Zhang et al., 2019)stated that by increasing the activity of extracellular enzymes that break down carbon and change the bacterial community in the soil, salinity could reduce the total amount of O.M. in the soil.

The application (S+BC) resulted in a substantial increase in organic matter, with levels rising from 63 to 96 in S-1 and from 61 to 77 in S2. Furthermore, the combination of (S+NP+BC) led to elevated organic matter levels, increasing from 75 to 87 in S-1 and from 67 to 82 in S2. In the P+NP group, organic matter content increased from 62 to 84 in S-1 and from 62 to 71 in S2. Additionally, the P+BC treatment led to a notable increase in organic matter, with levels rising from 68 to 89 in S-1 and from 65 to 78 in S2. The highest levels of organic matter were observed in the P+NP+BC group, reaching 92 in S-1 and 79 in S2. These results unequivocally highlight the effectiveness of biochar and its combinations with zinc nanoparticles in augmenting organic matter content in saline soils, thereby significantly enhancing soil health and resilience.

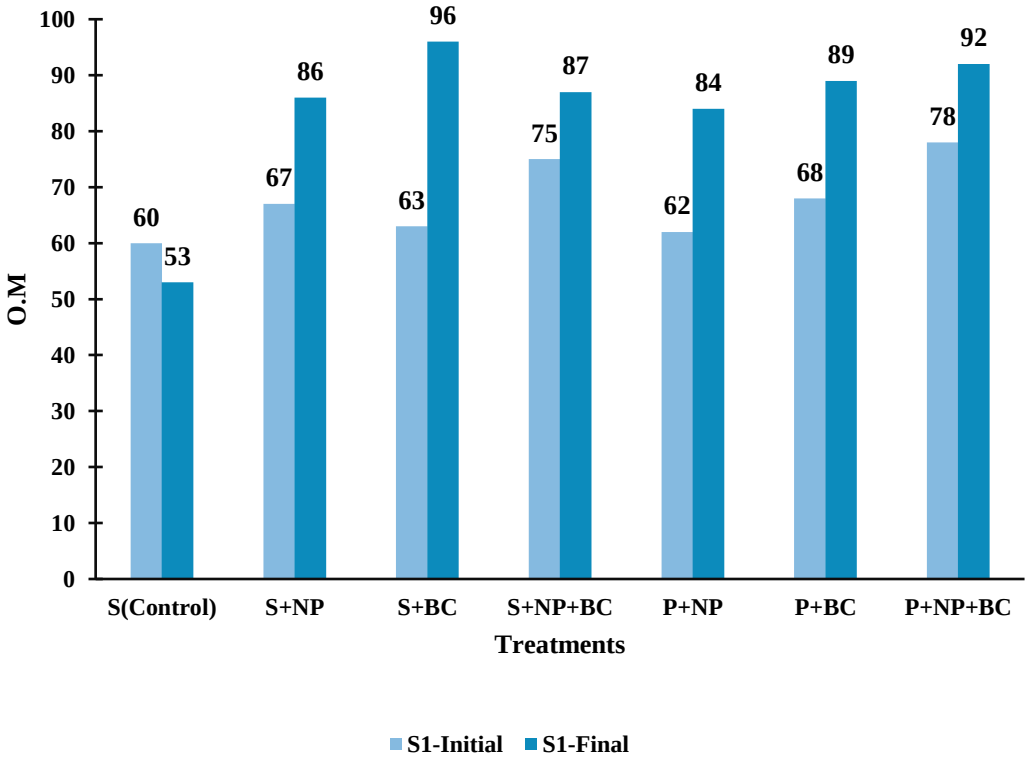


Figure 4.18(a) Results of organic matter in S-1 .

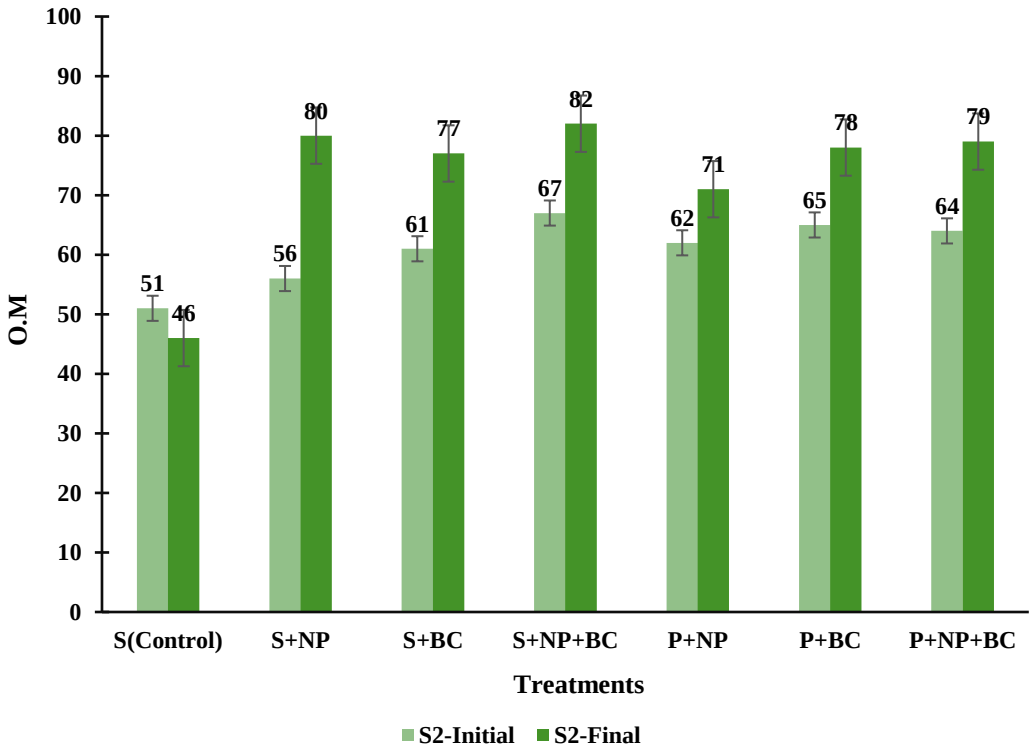


Figure 4.18(b) Results of Organic matter in S-2.

4.4 Plant analysis.

To monitor plant changes, plant analysis was conducted after 10 days of treatment and the final testing was done on day fifteen.

4.4.1 Proline

The proline content results in maize under salt stress with various treatments revealing the plant's adaptive responses to mitigate stress in (S-1 and S2 respectively). Proline is a key Osmo protectant in under stress plant conditions, often accumulating as a protective mechanism. In S1(control), proline levels increased from 0.55 to 0.61 µmol/g FW, indicating that the plant was responding to salinity stress by accumulating

proline. In S2(control), proline was measured at 0.59 and increased to 0.65 µmol/g FW. This suggests that proline accumulation was less effective, and degradation occurred over time as stress levels may have surpassed the plant's tolerance threshold.

In the P+NP treatment, the combination of plants with zinc nanoparticles resulted in a decrease in proline levels in both S-1 and S2. In S1, proline decreased from 0.41 to 0.17 µmol/g FW, while in S2, it dropped from 0.42 to 0.15 µmol/g FW. This decline suggests that zinc nanoparticles helped alleviate salt stress, reducing the need for proline accumulation. The treatment with ZnONPs significantly enhanced the proline content in the leaves of plants, regardless of the concentrations and durations. Notably, the roots of stock plants treated with ZnO-NPs exhibited the highest proline content, showing a remarkable 65.0% increase evaluated to the control and other treatments.

In P+BC, proline levels showed a moderate decrease in S-1, dropping from 0.44 µmol/g FW to 0.21 µmol/g FW, and in S2 from 0.43 to 0.23 µmol/g FW. Biochar likely improved soil properties and reduced salt stress, lowering proline accumulation as the plant experienced less osmotic stress. After 15 days, there was a noticeable drop in proline levels in the samples treated with biochar. This shows that biochar helps plants maintain better water status and lowers osmotic stress, which in turn lowers the demand for proline production (Wu et al., 2023).

In the P+NP+BC treatment, In S-1, proline dropped from 0.43 µmol/g FW to 0.16 µmol/g FW, and in S2, it decreased from 0.45 to 0.21 µmol/g FW. Results indicated that proline contents were decreased in BC-treated plants, possibly because of decreased ROS production and decreased antioxidant and osmotic stresses in biochar-treated plants (Kul et al., 2021). It was noted that the incorporation of ZnO NPs led to a reduction in proline content across all treatments. Specifically, the proline levels in plants treated with ZnO NPs were lower than those treated with bulk ZnO, as well as their corresponding control groups (Alabdallah & Alzahrani, 2020). This suggests that the combined treatment was the most effective at alleviating stress, reducing the plant’s reliance on proline as an Osmo protectant.

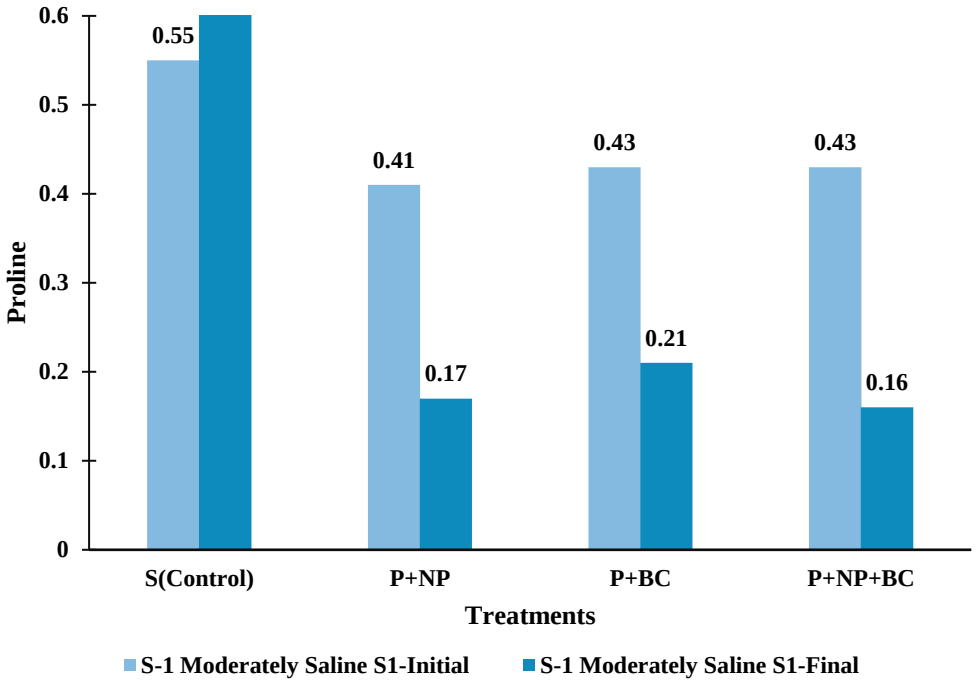


Figure 4.19(a) Results of proline in plants S-1.

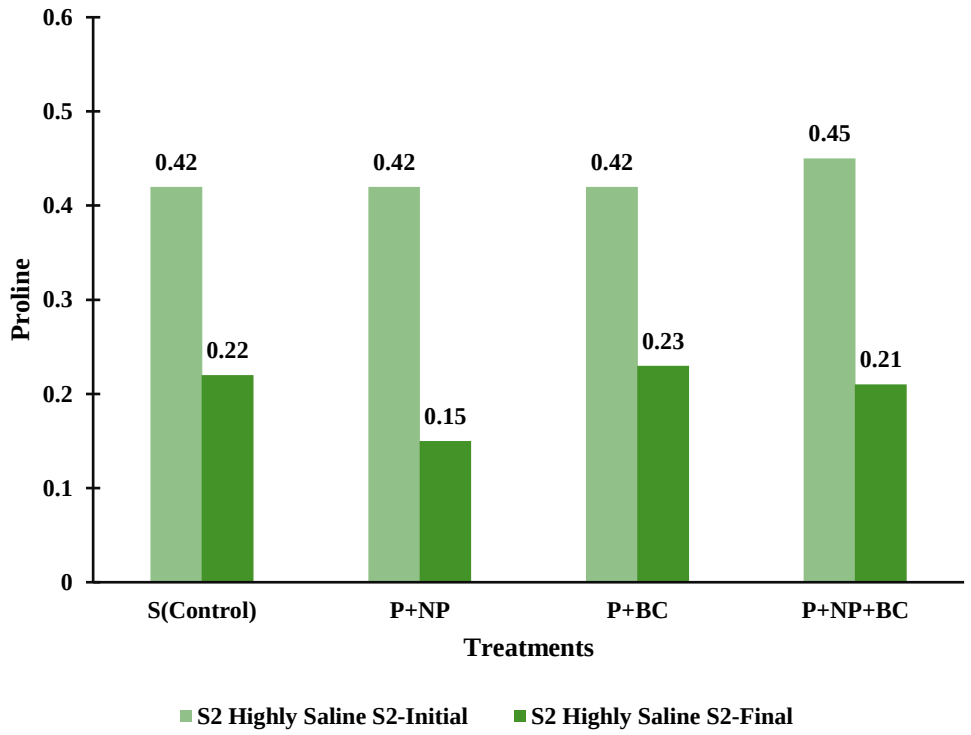


Figure 4.19(b) Results of proline in plants S-2.

4.4.2 Biomass

The analysis of biomass revealed significant differences in maize plants under salt stress across various conditions. The control group showed minimal biomass of 0.3 g in both saline conditions, indicating a severe unfavorable impact of salt stress on plant growth without treatment intervention. The P+NP treatment resulted in a notable improvement in biomass to 1.5 g in S1 and 2.2 g in S2, potentially due to the role of zinc nanoparticles in enhancing antioxidant defenses. Biomass levels for the P+BC treatment was 1.7 g in S1 and 1.6 g in S2. The combined treatment of P+NP+BC exhibited the highest biomass values, with 2.9 g in S1 and 3.3 g in S2, resulting in improved growth and biomass accumulation.

Table 2.4.4.2 Biomass of Plants

	Biomass (g)	
	S-1	**S2**
	Moderately Saline	*Highly Saline*
Treatments	***Initial***	***Final***
S(Control)	0.3	0.3
P+NP	1.5	2.2
P+BC	1.7	1.6
P+NP+BC	2.9	3.3

4.4.3 Antioxidant enzymes

Plants that are exposed to harsh environments, such as salt stress, require the presence of antioxidant enzymes to effectively manage oxidative stress exposed to high salinity levels often experience oxidative stress due to increased reactive oxygen species (ROS) production. If not controlled, these ROS can cause cell damage. By

neutralizing ROS, antioxidant enzymes aid in the mitigation of this damage. Plants under salinity stress experience increased ROS production and oxidative damage.

4.4.3.1 Catalase

An antioxidant enzyme that is essential for shielding cells from oxidative damage is catalase. In plants, catalase is essential for preserving the equilibrium of hydrogen peroxide within cells(I. Sharma & Ahmad, 2014).Under stress conditions, hydrogen peroxide tends to be released more. The catalase enzyme activity in maize was measured under different salt stress treatments. Distinct trends were observed in both moderately saline (S-1) and highly saline (S-2) conditions. In S1(control), catalase activity slightly increased from 0.00213 to 0.00228 µmol/g FW. Similarly, in S2, it decreased from 0.000456 to 0.000137 µmol/g FW. These decreases suggest that the plant's built-in defense mechanism was inactivated to manage (ROS) under stress. The activity of catalase (CAT) in control plants was minimal, while plants treated with ZnO-NPs exhibited the highest activity of these enzymes. The activity of Catalase increased by 69.7% in the treated plants (Faizan et al., 2021).

In the P+NP presence of zinc nanoparticles, catalase activity in S-1 was significantly enhanced, increasing from 0.0188 to 0.00441 µmol/g FW. However, the final value indicates a decline after the initial boost. In S-2, the enzyme activity was initially high at 0.136 but decreased to 0.041 µmol/g FW. This suggests that although zinc nanoparticles initially boost ROS-scavenging activity, prolonged exposure may lead to a decrease in catalase activity as stress is reduced, or the plant resorts to alternative antioxidant mechanisms. There was a rising trend in the activity of antioxidant enzymes like CAT in the plants that received ZnO-NPs treatment compared to the control plants. The plants exposed to ZnO-NPs along with NaCl showed the highest increase in CAT activity, which was 57% indicating NPs were evident in activating catalase antioxidant activity because it can be suppressed because of saline conditions (Faizan et al., 2021).

The P+BC biochar treatment unequivocally led to a decline in catalase activity in S-1 from 0.0141 to 0.00151 µmol/g FW, clearly indicating that biochar reduced stress-induced ROS production, resulting in a lower demand for catalase. Conversely, in S2, the enzyme activity increased from 0.137 to 0.144 µmol/g FW, showing that biochar effectively maintained or even slightly enhanced the plant's defense system under highly saline conditions, by using biochar, antioxidant activity catalase is also improved, maintaining membranes from the detrimental effects of salinity (Kavitha et al., 2018).

In the P+NP+BC combination, using zinc nanoparticles and biochar distinctly increased catalase levels in S-1 to 0.0654 µmol/g FW, followed by a decline to 0.00304 µmol/g FW. Zinc nanoparticles have been shown to significantly lower catalase activity, which implies that they may be able to reduce oxidative stress and improve plant ability to tolerate salinity (Seleiman et al., 2023). In S2, catalase activity remained robust and stable, at 0.137 µmol/g FW to 0.138 µmol/g FW. This unequivocally suggests that the combined treatment effectively managed ROS at an optimal level without excessive catalase activity, showing a balance between ROS production and scavenging. The data undeniably demonstrates a substantial increase in catalase (CAT) activity as salt concentrations escalated, peaking at 75% and then declining at 100%. It was evident that the introduction of bulk ZnO resulted in lower CAT activity compared to ZnO nanoparticles (NPs). Importantly, there was a noteworthy increase in CAT activity in plants treated with ZnO NPs in comparison to their respective controls (Alabdallah & Alzahrani, 2020).

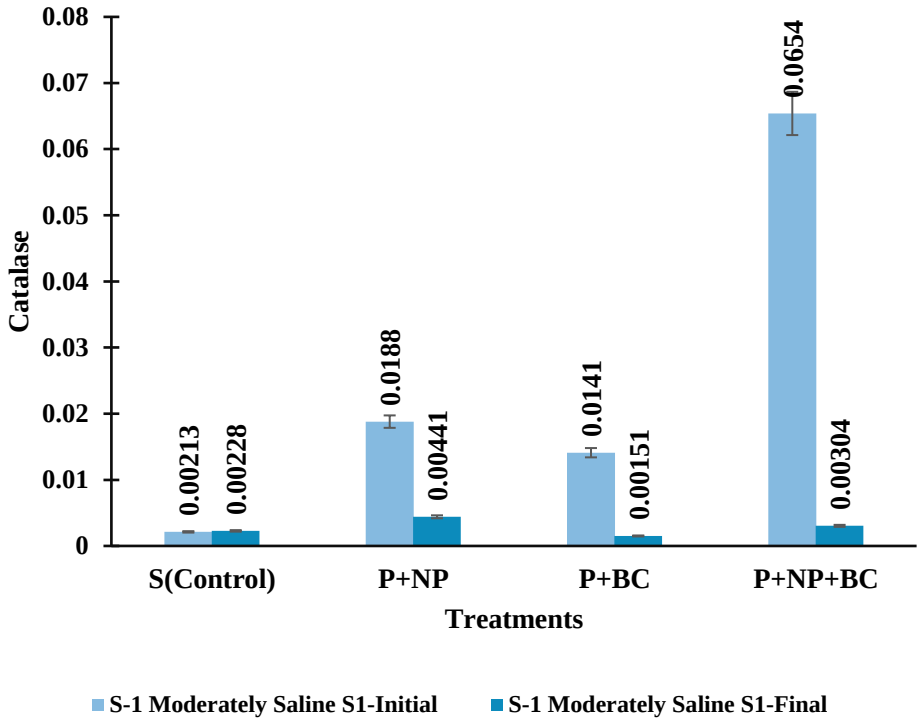


Figure 4.20(a) Results of CAT in plants S-1

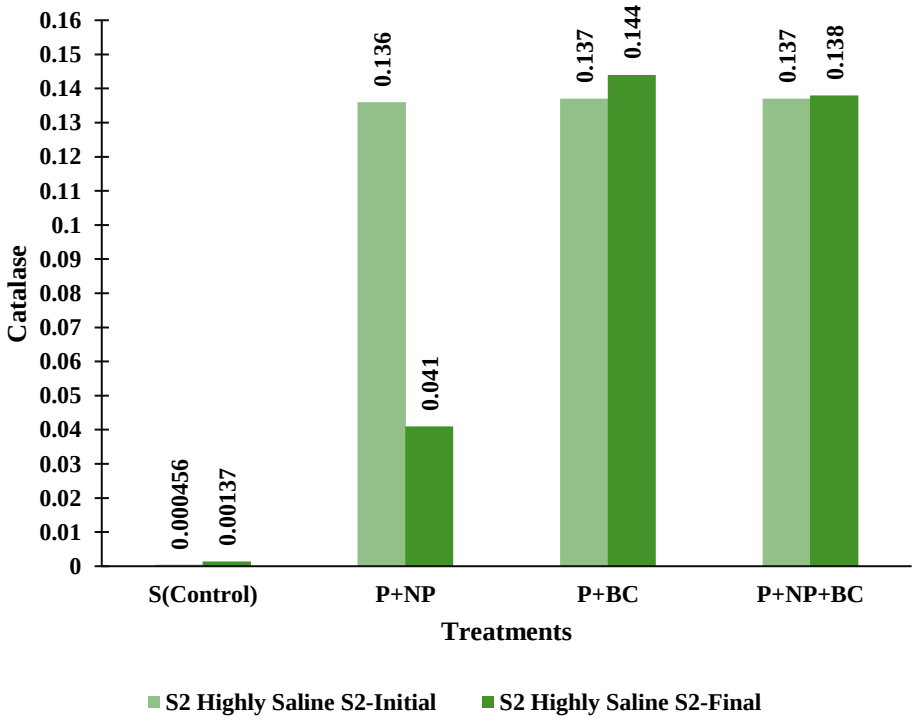


Figure 4.20(b) Results of CAT in plants S-2

4.4.3.2 Peroxidase

Reactive oxygen species (ROS) like hydrogen peroxide ($H2O_2$) are broken down into water by peroxidase (POD). Peroxidase transfers electrons from a variety of organic and inorganic electron donors, such as phenolic compounds, ascorbate, and guaiacol, to H_3O_2, in contrast to catalase, which uses H_2O_2 directly. The peroxidase activity in maize under salt stress varied across different treatments, reflecting the plant's antioxidative defense mechanisms in both S-1 and S2 conditions. In the S-1, peroxidase activity decreased from 2.478 to 1.436 µmol/g FW, suggesting that sustained stress diminishes the plant's ability to maintain high peroxidase levels. Similarly, in the S2, the activity slightly declined from 1.572 to 1.436 µmol/g FW, indicating that the plant's natural defense system is unable to maintain elevated peroxidase activity without external treatment.

The application of zinc nanoparticles (P+NP) led to a significant decrease in peroxidase activity from10 to 15 days in both S-1 from 0.302 to 0.216 µmol/g FW and S2 from 0.821 to 0.488 µmol/g FW. The highest levels of peroxidase (POD) in tubers were observed in T5 (ZnO NPs) treatment, while the minimal levels were detected in the natural plants (T0) used as the control. In comparison to the control, the highest POD activity in potato tubers was recorded in the ZnO treatment(Mahmoud et al., 2020).Similarly, the P+BC reduced peroxidase activity in S-1 from 1.472 to 0.507 µmol/g FW and significantly in S2 from 0.702 to 0.096 µmol/g FW, possibly by improving soil structure and nutrient availability, and thereby lessening oxidative stress. Biochar reduced the peroxidase content under salinity stress (Fazal & Bano, 2016)). The combination of P+NP+BC demonstrated a more complex response. In S-1, peroxidase activity slightly increased from 0.311 to 0.851 µmol/g FW, possibly indicating a synergistic effect that optimized the plant's defense mechanism. In S2, the peroxidase activity increased significantly from 0.855 to 1.572 µmol/g FW, suggesting that this combination was highly effective in stimulating the plant's antioxidative defenses under more severe saline conditions.

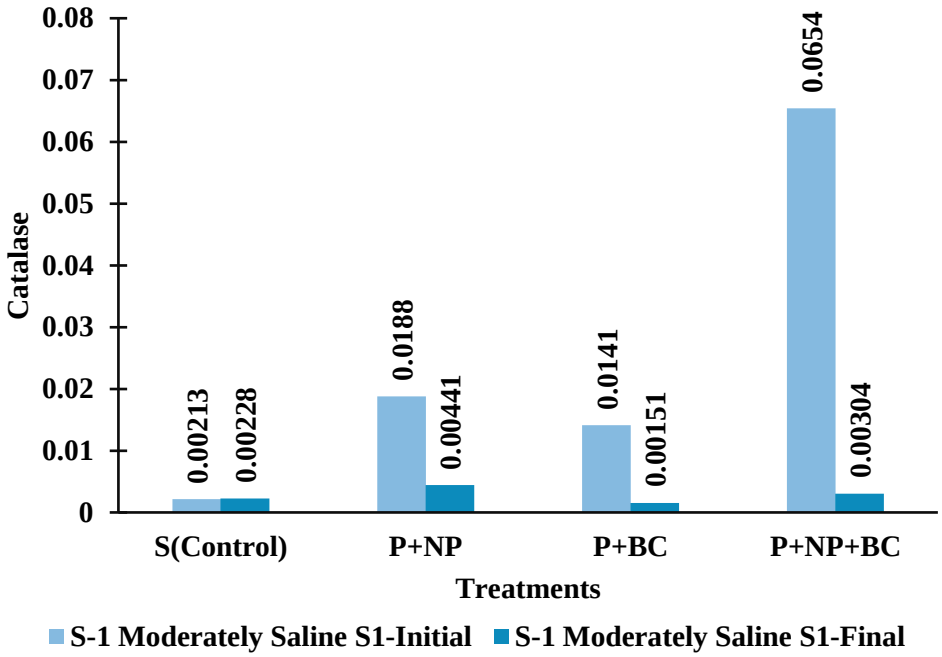


Figure 4.21(a) Results of POD in plants S-1

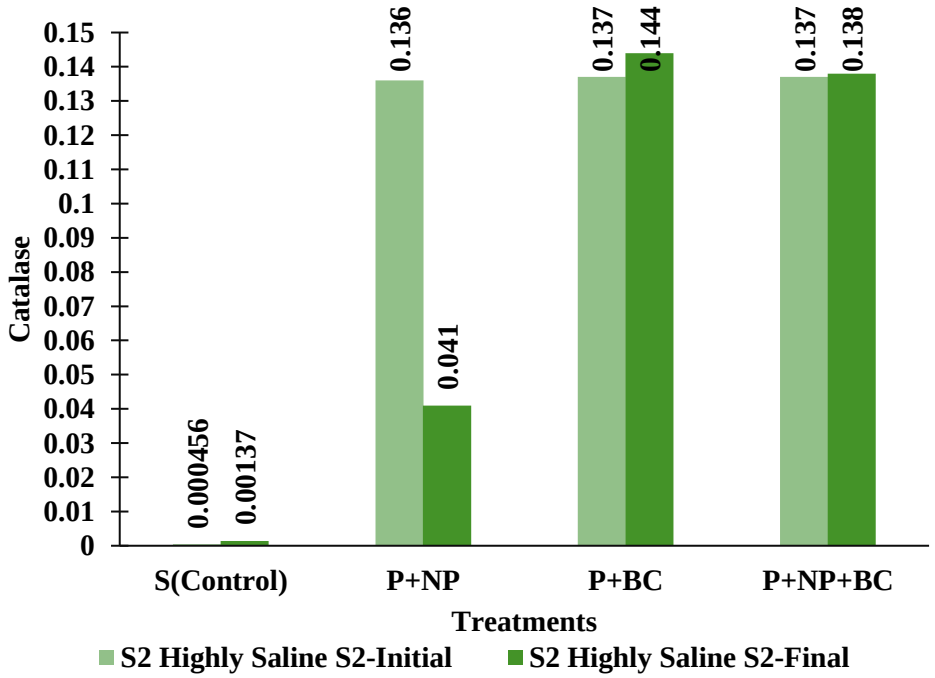


Figure 4.21(b) Results of POD in plants S-2

4.5 Zinc analysis of soil

After treating saline soil with zinc oxide nanoparticles, the zinc concentrations varied under different treatments for moderate saline soils (S-1) and highly saline soils (S2). In the control, zinc concentrations were low in both soil types (1.25 ppb in S-1 and 0.98 ppb in S2), indicating insufficient baseline zinc levels in untreated saline soils. Following treatment with zinc oxide nanoparticles, zinc concentrations increased to 2.02 ppb in S-1 and 2.01 ppb in S2 for the S+NP treatment. This increase was expected due to the direct addition of zinc nanoparticles, which can enhance the bioavailability of zinc. In the P+NP treatment, zinc levels were lower compared to other nanoparticle treatments, measuring 0.98 ppb in S-1 and 1.39 ppb in S2.

For the S+BC treatment, zinc concentrations were 1.89 ppb in S-1 and 1.25 ppb in S2. This treatment likely enhanced zinc retention by binding to zinc ions and reducing leaching, increasing the zinc content in the soil. In the P+BC treatment, zinc concentrations moderately increased to 1.27 ppb in S-1 and 1.59 ppb in S2. The combined S+NP+BC treatment increased zinc concentrations to 1.85 ppb in S-1 and 3.09 ppb in S2, showing the synergistic effect of biochar and nanoparticles in retaining zinc in highly saline soil. In the P+NP+BC treatment, zinc concentrations were moderate in both soil types, measuring 1.1 ppb in S-1 and 1.89 ppb in S2.

Regarding the normal amount of zinc required in soil, the analysis aimed to determine whether the levels of zinc in the soil after ZnO NP applications exceeded the levels that could make the soil toxic. The typical range of zinc in soil is 0.5 to 2.0 ppm (500-2000 ppb), depending on soil type and crop requirements. Further enhancement is needed to meet the optimal requirements for most crops, as the levels observed in these treatments are relatively low (Alloway, B.J.2008).

Table 3.4.5.1 Zinc Analysis of soil after applying ZnO Nanoparticles

Zinc (ppb)		
	S-1	**S2**
	Moderately Saline	*Highly Saline*
Treatments	**Final**	**Final**
S(Control)	1.25	0.98
S+NP	2.02	2.01
S+BC	1.89	1.25
S+NP+BC	1.85	3.09
P+NP	0.98	1.39
P+BC	1.27	1.59
P+NP+BC	1.1	1.89

CONCLUSION

- The production of ZnO nanoparticles was confirmed through SEM, FTIR, and XRD analyses, showing a mesoporous wurtzite structure with typical Zn-O vibrational peaks and characteristic XRD diffraction patterns.

- ZnO nanoparticle reduced salinity in soil by lowering both pH and electrical conductivity (EC), with significant reductions in S1 (pH 8.95 to 7.82, EC 5.08 to 4.15 ds/m) and S2 (pH 8.81 to 7.49, EC 10.95 to 8.92 ds/m).

- The addition of ZnO nanoparticles, BC, and nano-biocomposite increased nutrient levels in the soil, particularly nitrogen (N), phosphorus (P), and potassium (K), showing significant increases, especially in moderately saline soils (e.g., N increased from 11.83 to 64.66 mg/kg in S1).

- The application of ZnO nanoparticles also enhanced antioxidant activity (catalase, peroxidase) and proline content, contributing to better stress resistance in plants under saline conditions.

- Results were evident in showing the potential of treatments, whereas the effectiveness of treatments “ZnO NPs > ZnO NPs + BC > BC” in improving soil capacity to withstand salt stress and aiding plants in survival in stress conditions.

RECOMMENDATIONS

1. Further research should be conducted to examine the impacts of nanobio composite interaction with saline soil.
2. The same approach should be used with various common agricultural plants.
3. To gain a deeper understanding of how soil types interact with nanoparticles, it is important to use the same setup when treating different types of saline soil.
4. Various concentrations of ZnO nanoparticles and biochar should be applied to determine the optimal concentrations.
5. This setup should also be tested on a small area of agricultural land to assess its impact, limitations, and effectiveness in a completely natural environment.

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