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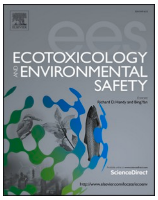

Improving wheat physio-biochemical attributes in ciprofloxacin-polluted saline soil using nZVI-modified biochar

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ABSTRACT

The Ciprofloxacin (CIP) toxicity and salinity stress in agricultural soils cause risk to environmental and food safety. Consequently, it is essential to devise or use more effective techniques for mitigating salinity and ciprofloxacin-induced stress in soil. This study includes the nZVI-loaded biochar synthesis, integrating the unique characteristics of raw biochar with nZVI. The present study examined the impact of raw and nZVI-loaded biochar on soil quality and the mitigation of salinity stress and Ciprofloxacin toxicity in wheat plants. The results showed that the application of nZVI-loaded biochar treatments led to substantial enhancement in shoot biomass, root biomass, grain biomass, and spike biomass by 152.1, 54.3 %, 59.8 %, and 151 %, respectively compared to control treatment. The treatment with nZVI-loaded biochar significantly increased the rates of photosynthesis and transpiration, as well as the conductance of stomata. It also resulted in higher levels of intercellular CO_2, photosynthetic pigments, and water use efficiency with increases of 49 %, 59 %, 57 %, 37 %, 40 %, and 95 %, respectively. The nZVI-loaded biochar significantly decreased electrolyte leakage, malondialdehyde (MDA), and hydrogen peroxide levels compared to the NaCl treatment alone. It also enhanced the activities of enzymatic antioxidants such as peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), and ascorbate peroxidase (APX). The non-enzymatic antioxidants including total soluble sugars (TSS), flavonoids, total soluble proteins (TSP), phenolics, ascorbic acid, anthocyanin, proline, and glycine betaine

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significantly influenced by the nZVI-loaded biochar. The nZVI-loaded biochar effectively alleviates the stress of soils that are contaminated with hazardous amounts of Ciprofloxacin while improving the soil and plant health.

1. Introduction

The deterioration of the environment has increasingly constrained world crop production (Khondoker et al., 2023). The productivity of crops is considerably influenced by abiotic factors and stresses, including harmful organic contaminants for example Ciprofloxacin (CIP) and other antibiotics, soil salinity, and heavy metal contamination (Akensous et al., 2022). Ciprofloxacin (CIP) is a very harmful environmental pollutant that mostly penetrates agricultural soils through the Animal excrement elimination, industrial processes, Excrement produced by humans, Wastewater generated by hospitals, and Runoff originating from regions where manure is stored on the surface (Kong et al., 2024). Ciprofloxacin exhibits significant mobility in the environment, rendering it susceptible to bioaccumulation in the food chain via plants. This may result in significant disturbances in the health of living organisms (Khan et al., 2024). Previous studies have shown that ciprofloxacin is very toxic to plants, hindering their growth and perhaps resulting in crop death (Gahrouei et al., 2024). Study carried out by Gomes et al. (2019) has demonstrated that Ciprofloxacin toxicity has a detrimental effect on the process of photosynthesis and the absorption of essential minerals, as well as negative impacts on growth of maize plants. Furthermore, Ciprofloxacin triggers oxidative-stress in crops via increasing the generation of ROS such as electrolyte leakage, hydrogen peroxide, and malondialdehyde (Yan et al., 2019). Excessive generation of ROS can harm large molecules that make up living organisms and disrupt the system that protects against oxidative damage in plants (Marques et al., 2021).

Conversely, over 800 M ha of land globally are impacted thru salt stress (Tessema et al., 2022). The primary cause of soil degradation in agriculture is salinity resulting from the use of wastewater, phosphate-based fertilizers, sewage sludge, and biosolids for irrigation (Pandey et al., 2024). Research has demonstrated that exposure to high amount of salt can cause to lessening in plant growth, biomass, and the absorption of essential mineral nutrients (Ondrasek et al., 2022). Furthermore, under salt stress led in reduction of calcium and potassium concentrations in wheat seedlings, as seen in a study conducted by (Shabaan et al., 2022). In high salinity levels induced oxidative stress in wheat, leading to a decrease in activity of antioxidant enzymes (Attia et al., 2023).

Wheat (*Triticum aestivum*) is a vital constituent of the human food-chain and provides the primary source of sustenance for the majority of the global population. In 2023, Asia produced over 330 MT of wheat; whereas the global wheat production was reported to be around 787.4 MT. Pakistan's wheat production in 2023 amounted to approximately 29.69 million tons (Chauhdary et al., 2024). Several studies have indicated that contaminants have the potential to accumulate in wheat and can be passed on to food-chain through use of the wheat-based products (Sikandar et al., 2024). Furthermore, wheat has the ability to thrive in subpar soils, making it a viable option for feeding the growing population. Additionally, the scarcity of high-quality irrigation has led to the use of sewage water for irrigating wheat crops (Zawar et al., 2024; Sindesi et al., 2023). In actual field conditions, soils that primarily receive sewage sludge and wastewater are frequently exposed to various stresses, including salt, heavy metals, and other contaminants (Muhae-Ud-Din et al., 2024). Saline soils exhibit reduced fertility and possess a high degree of bioavailability of contaminants (Gantayat and Elumalai, 2024). The simultaneous existences of salinity and contaminants in soils have a detrimental impact on crops, which is more severe compared to the individual effects of these stresses (Selvam et al., 2024; Fatemi et al., 2023). For instance, the separate application of contaminants and NaCl, as well as their combined application, resulted in lessening in plant growth, relative water content (RWC), and chlorophyll level (Awasthi et al., 2022). The combined application of NaCl and contaminants resulted in a reduction in plant height, length of roots, MDA level, and activity of the antioxidant enzymes in wheat than individual treatment of contaminants or NaCl alone (Guan et al., 2024). The administration of sodium chloride (NaCl) on the leaves of wheat resulted in an enhanced build-up of contaminants than control treatment, as reported by Farhad et al. (2024). According to Wan et al. (2024), the halophyte species *C. rossii* showed a greater accumulation of Cd concentration in its shoots when exposed to both salt stress and contaminants stress, compared to when exposed to either salt stress or contaminants stress alone. The concurrent exposure to NaCl and contaminants resulted in an elevation of the MDA levels in corn seedlings, as compared to the individual exposure to contaminants stress alone (Zuo et al., 2021).

Various approaches have been devised to mitigate the detrimental impacts of contaminants and salt stressors on plants (Lee and Kasote, 2024). Biochar, an organic substance produced through pyrolysis with limited oxygen supply, is currently receiving significant attention as a soil supplement globally (Amalina et al., 2023). The amendment of biochar enhances SOC, mends the fertility of the soil, as well as mitigates soil pollution caused by pesticides and contaminants, owing to its excellent adsorption capabilities (Shoudho et al., 2024). However, the adsorption of contaminants on the biochar surface is restricted because of the lower specific surface area and reduced number of negative charges on the biochar surfaces. Prior research has indicated that the application of unaltered biochar reduces the harmful effects of contaminants on plants to a certain degree, while also enhancing soil quality and promoting plant growth (Dutta et al., 2024). Applying metal salts/minerals onto the surfaces of biochar can improve biochar's ability to adsorb hazardous materials (Beygisangchin et al., 2021). Modified/engineered biochar can effectively reduce the availability and solubility of contaminants in soil by influencing the chemical forms, uptake, precipitation, and formation of complexes with contaminants (Patel et al., 2021). It is essential to create modified/engineered biochar as an effective, stable, cost-effective, and environmentally friendly solution for improving soils contaminated with contaminants, in line with the principles of sustainable development. This study addressed the synthesis process of composite material through the doping of nano zero-valent iron (nZVI) onto biochar. This study explored the effects of untreated biochar and biochar enhanced with nZVI on soil quality, as well as their effectiveness in alleviating salt stress and ciprofloxacin toxicity in wheat plants.

2. Methodology

2.1. Collection of soil samples and generation of raw biochar and nZVI-loaded biochar

Soil used in the present research was collected from an agricultural field of Islamia University of Bahawalpur, Punjab, Pakistan. The soil used is primarily dedicated to the growing of wheat, with farmers predominantly relying on untreated urban wastewater as a substitute for canal water due to its scarcity for irrigation purposes. Consequently, the soil has become contaminated with hazardous substances, mainly Ciprofloxacin (CIP), due to the continuous discharge of untreated urban wastewater for over two decades. The soil is composed of sandy loam, has an alkaline pH, and does not include any carbonates. Soil samples were collected from the top layer using a stainless-steel spade. They were then dried in the shade, crushed into small pieces, and passed through a sieve with a 2-mm mesh size. For an in-depth scrutiny of the characterization techniques and soil attributes, followed Garrison and Garrison (2016). Total ciprofloxacin levels in the soil were determined using 1 g of soil that had been dried in the air, mixed with 10 mL of concentrated HNO_3, and left in flasks overnight. The solution was heated to 200°C, solution was cooled. Then, an additional 1 mL of HNO_3 and 4 mL of Perchloric acid was incorporated into the mixture and mixture was heated. The samples were extracted from hot-plate once fumes of Perchloric acid became apparent. Subsequently, the samples were cooled and treated with a solution of 1:10 hydrochloric acid. The samples were then heated to a temperature of 70 °C for 1 hour, followed by another cooling period. To achieve a final volume of 50 mL, the samples were diluted with a 1 % hydrochloric acid solution. Finally, the samples were filtered with Whatman filter-paper No. 42 (John et al., 2006). Bouyoucos (1962) technique was employed to determine particle size of soil, while pH of soil-saturated paste was determined using pH meter (M22). Electrical conductivity (ECe) and soluble ion measurements and Na adsorption ratio were conducted using established procedures by Page et al. (1982). To determine the quantities of available metals and Ciprofloxacin in the soil, a 10 g sample of soil that had been dried in the air was extracted using a solution of ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) with a pH of 7.6, as described by Soltanpour (1985). Soil physicochemical parameters were determined are documented in Table 1.

2.2. Preparation of raw biochar and nZVI-loaded biochar

The corncob biochar underwent pyrolysis at temperatures of 400 °C. The biochar was pulverized and passed through a 0.25 mm plastic sieve. Tea polyphenols (TP) are used as an eco-friendly, and inexpensive reducing agent to create nZVI. This process results in the production of nZVI with strong antioxidant capabilities. The technique for preparing nZVI-loaded biochar is explained as follows. Initially, a specific amount of $FeSO_4 \cdot 7\ H_2O$ was added to 100 mL of water, ensuring that it completely dissolved. Afterward, 0.2 g of biochar was added to the beaker and then agitated for 1 hour. Subsequently, a quantity of 1.2 g of tea polyphenols was incorporated into the combination of $FeSO_4 \cdot 7\ H_2O$ and biochar and agitated for 1 hour. The obtained mixture underwent solid-liquid separation through the utilization of a vacuum pump. The solid portion was subjected to drying in a drying box at temperature of 65 °C for 12 hours. After the process of drying, the solids were carefully crushed and filtered through a 60-mesh sieve, resulting in the creation of the desired powdered product known as nZVI-biochar. The preparation processes of nZVI-loaded-biochar as shown in Figure S1. The physiochemical properties of raw and nZVI-loaded-biochar are presented in Table 2.

Table 1
Soil physicochemical traits used in experiment.

Texture of soil	Sandy clay loam
Clay %	32
Silt %	24
Sand %	44
pH	7.29
CEC ($cmol_c kg^{-1}$)	5.03
EC (dS m^{-1})	3.04
BD (gcm^{-3})	1.49
OM %	0.73
OC%	1. 23
Avail. P (mg kg^{-1})	2.84
Avail. K (mg kg^{-1})	11.64
SOM (g kg^{-1})	20.34
Total N %	0.17
Total P %	0.004
Total K %	0.03
CO_3 (mmol l^{-1})	2.75
Cl^- (mmol l^{-1})	4.42
HCO_3 (mmol l^{-1})	6.61
K^+ (mmol l^{-1})	0.10
Na^+ (mmol l^{-1})	3.12
$Ca^{2+} + Mg^{2+}$ (mmol l^{-1})	16
Total Cd (mg kg^{-1})	2.90
Available Cd (mg kg^{-1})	0.39
Total Zn (mg kg^{-1})	48.53
Available Zn (mg kg^{-1})	5.49
Total Mn (mg kg^{-1})	70.65
Available Mn (mg kg^{-1})	9.32
Total Ni (mg kg^{-1})	5.29
Available Ni (mg kg^{-1})	0.59
Total Ciprofloxacin (mg kg^{-1})	15.61
Available Ciprofloxacin (mg kg^{-1})	4.32
Total Enrofloxacin (mg kg^{-1})	8.63
Avail. Enrofloxacin (mg kg^{-1})	1.34
Total Fleroxacin (mg kg^{-1})	6.39
Available Fleroxacin (mg kg^{-1})	1.52
Total Sulfadiazine (mg kg^{-1})	7.33
Available Sulfadiazine (mg kg^{-1})	1.43
Total Tetracycline (mg kg^{-1})	8.11
Avail. Tetracycline (mg kg^{-1})	1.76
Total Sulfadimidine (mg kg^{-1})	7.54
Available Sulfadimidine (mg kg^{-1})	1.98

2.3. Experimental setup

A pot experiment conducted in greenhouse of Islamia University of Bahawalpur, Punjab, Pakistan, during the Rabi season from Nov. 2023 to April 2024, in ambient environmental circumstances. Every pot, with a height of 20 cm and top and bottom diameters of 18 cm and 15 cm were filled with 2.5 kg soil (air-dried). A 5 % w/w dose of raw and nZVI-loaded biochar was administrated into soil and mixed properly. The soil was then incubated for 2 weeks at 40 % of water-holding capacity (WHC). Before planting, wheat (Dilkash-20) seeds were sterilized using a 10 % (v/v) solution of H_2O_2 for 15 minutes and subsequently rinsed with distilled water. The chosen wheat type possesses the capacity to gather a low level of metals and toxic substances such as antibiotics in its shoots and exhibits a stronger ability to tolerate contaminants (Ahmad et al., 2023). At the start, 20 seeds were planted in each pot, and this process was repeated three times using a completely randomized design. After seven days, 10 plants were kept in each container following germination. After forty-five days from the time of planting, seedlings

Table 2
The physiochemical properties of raw and nZVI-loaded-biochar.

Parameters	Raw-biochar	nZVI-loaded-biochar
pH	7.21	10.87
Dry matter %	90.19	96.24
Volatile matter %	61.9	53.65
Moisture level %	6.87	4.11
EC (mS/cm)	13.25	13.19
CEC (cmol/kg)	250.31	232.29
SSA (m^2/g)	33.52	191.4
Pore volume (cm^3/g)	0.004	0.016
Pore diameter (nm)	22.300	17.210
Fe %	0.02	13.7
C%	58.21	52.19
O%	16.7	24.8
H%	3.79	1.49
S%	0.02	0.04
H/C	0.66	0.44
O/C	0.26	0.18
Fixed carbon %	30.79	27.65
N %	0.36	0.30
OC %	4.64	5.74
C:N	4.21	7.11
Total K %	2.22	2.23
Total P %	0.30	0.20
BD (g cm^{-3})	0.63	0.62
Ash %	0.48	0.53
Available P (mg kg^{-1})	0.37	0.64
Exchangeable Mg (cmol kg^{-1})	7.81	8.87
Exchangeable Ca (cmol kg^{-1})	4.49	4.76
Exchangeable K (cmol kg^{-1})	1.81	1.91

were exposed to 3 different amounts of saline irrigation treatments: 0, 20, and 40 mM NaCl. The saline levels were established by mixing NaCl into water with electrical conductivity values of 0.4 dS m^{-1}. The total volume of the water used in each pot was 2.5 Liters. This was achieved through mixing 400 mL of either saline-nature or non-saline water twice a week, with a total of 6 saline irrigations over three weeks. Saline irrigations ended due to inhibited plant growth in pots subjected to 5 % biochar + 50 mM NaCl stress. There was a total of 9 treatments in the completely randomized design, with 3 replicates of each treatment. The control group (0 mM NaCl + 0 % biochar) did not receive any treatment including the biochar and salinity application.

2.4. *Harvesting of biomass assessment*

Plants were collected at the physiological maturity, which occurred subsequently 130 days after germination. It was subsequently separated into its shoot, root, and spikes. The lengths of the spike and shoot were determined using a ruler. Subsequently, the plant parts were meticulously rinsed with tap water, and then further cleansed with distilled water. The root samples were cleansed using a 1 % hydrochloric acid solution, followed by a thorough rinse with distilled water. Samples were further separated into various parts, including roots, grains, and shoots. An oven was used to dehydrate samples at a temperature of 70 °C until a consistent weight was achieved. The dry biomass of each component was then measured individually, crushed, and kept for the next analysis.

2.5. *Parameters related to gas exchange and photosynthetic pigments*

After 65 days of seeding, the levels of chlorophyll and gas exchange characteristics were assessed by removing one plant from each treatment and replicating. The chlorophyll levels were quantified with spectrophotometer (DR3900) at different wavelengths given formulas in Eqs. 1, 2, and 3. Before measurement, samples were extracted with 85 % (v/v) acetone in absence of light at temperature of 4°C for 24 hours. Subsequently, samples were centrifuged at a speed of 4000×g for 10 minutes (Gitelson and Merzlyak, 1997). Gas exchange parameters such as water use efficiency (WUE), transpiration rate (Tr), photosynthetic rate (Pn), and stomatal conductance (Gs) and intercellular carbon dioxide concentration (Ci) were assessed using a portable infrared gas analyzer (IRGA). These factors were determined between 10a.m. and 12a.m. when plants were completely functional.

$$Chla = [(12.7 \times O.D663) - (2.69 \times O.D645)] \times V/1000 \times W \tag{1}$$

$$Chlb = [(22.9 \times O.D645) - (4.68 \times O.D663)] \times V/100 \tag{2}$$

$$Total\ Chl = [(20.2 \times O.D645 + (8.02 \times O.D663)] \times V/1000 \times W \tag{3}$$

2.6. *Assessment of EL, and reactive oxygen species (ROS)*

After sixty days from planting, the EL levels in the shoots were measured through removing one plant from each replication and treatment. Samples were separated into small pieces and positioned vertically in tubes containing 7 mL of DI water. Initial electrical conductivity of solution was measured after incubating tubes at temperature of 32°C for 2 hours. Samples were subjected to autoclaving for duration of 20 minutes at a temperature of 120 °C. The final electrical conductivity of the solution was measured after sample was cooled to 25 °C. The EL was computed with equation provided by Dionisio-Sese and Tobita (1998).

$$EL = (EC_1/EC_1) \times 100 \tag{4}$$

The Malondialdehyde (MDA) levels were determined using the technique outlined through Davey et al., (2005).

To test concentration of H_2O_2 (Hydrogen peroxide), a solution of phosphate buffer (50 mM, pH 6.6) was mixed with 50 mg leaf samples to create uniform mixture. The mixture was then subjected to centrifugation at 6000×g for 30 minutes at a temperature of 4 °C. To test the concentration of H_2O_2, solution of phosphate buffer (H_2KO_4P) (50 mM, pH 6.5) was mixed with 50 mg leaf samples to create uniform mixture. The mixture was then subjected to centrifugation at 6000×g for 30 minutes at temperature of 4°C. Next, 1 mL of a solution containing 0.1 % titanium sulfate in 20 % (v/v) sulfuric acid was added to extracted solution. The resulting combination was then subjected to centrifugation at a force of 6000 ×g for 20 minutes. The centrifugation was carried out at regulated temperature of 4°C. Supernatant's absorbance was quantified at a wavelength of 410 nm. The H_2O_2 concentrations were computed with extinction value of 0.28 $\mu mol^{-1}cm^{-1}$.

2.7. *Enzymatic antioxidants activities*

2.7.1. *Peroxidase (POD)*

Chance and Maehly (1955) technique was used to quantify peroxidase activity level. A cuvette was made by adding 0.05 mL of the sample extract, 7.5 mL of phosphate buffer, 0.1 mL of guaicol solution (composed of 335 µl of H_2O_2 and 15 µl of phosphate buffer), and 0.1 mL of H_2O_2 solution (composed of 100 µl of H_2O_2 and phosphate buffer 20 µl). The spectrophotometer employed to determine absorbance at 470 nm at intervals of 0, 30, 60, and 90 seconds.

2.7.2. *Superoxide dismutase (SOD)*

The measurement of SOD activity was conducted following the methodology outlined through Giannopolitis and Ries (1977). Reaction mixture consists of nitro-blue tetrozolium (50 µL), riboflavin (50 µL), L-methionine (100 µL), phosphate buffer (250 µL), tritox (100 µL), and DI water (150 µL). The sample was subjected to exposure for duration of 20 minutes, and the degree of light absorption was measured at a wavelength of 560 nm with spectrophotometer.

2.7.3. *Catalase (CAT)*

Chance and Maehly technique (1955) was used to quantify catalase level. At first, phosphate buffer (5 mL) was mixed with 0.2 g of finely crushed leaf sample. Subsequently, solution was subjected to centrifugation at speed of 1500 rpm for the 20 min, after which the supernatant was separated perpendicularly. Afterward, a cuvette was filled with 0.1 mL of sample, H_2O_2 (1 mL), and phosphate buffer (1.9 mL). The absorbance at 240 nm was determined using spectrophotometer at time intervals of 0, 30, 60, and 90 seconds.

2.7.4. *Guaiacol peroxidase (GPX)*

To measure guaiacol peroxidase activity, absorbance of a mixture reaction was recorded at 470 nm. Reaction mixture contained of 750 µL of 100 mM phosphate buffer (pH 7), 100 µL of 70 mM Hydrogen peroxide, and 750 µL of 10 mM guaiacol (Chance and Maehly, 1955). A guaiacol extinction value of 26.6 $mM^{-1}cm^{-1}$ was used to compute guaiacol peroxidase level.

2.7.5. *Ascorbate peroxidase (APX)*

The level of ascorbate peroxidase was measured with technique stated through Nakano and Asada (1981). The reaction mixture used to determine APX consisted of 200 µl of a 2 mM ascorbate solution mixed in a 100 mM phosphate buffer with 7 pH, 200 µL of a 10 mM hydrogen peroxide solution, 30 µL of a 5 mM EDTA solution, and protein extract (20 µL). The mixed reaction's absorbance was determined at wavelength of 290 nm, and APX level was measured with an extinction coefficient of 2.8 $mM^{-1}cm^{-1}$.

2.8. *Non-enzymatic antioxidants analysis*

2.8.1. *Glycine betaine (GB)*

To determine the glycine betaine content, 0.20 g of the fresh material was extracted using 5 milliliters of DI water. Sample was centrifuged at a speed of 15000 rpm for the 15 minutes. 500 µl of the resultant extract were combined with 1 millilitre of 2 N sulphuric acid and 1 millilitre of the sample extract in test tube. The test tubes were cooled for duration of 90 minutes after the addition of 0.2 mL of potassium tri-iodide. DI and 6 mL of 1,2dichloroethane were poured separately into the ice-cooled test tubes. Two separate layers were formed, and the lower layer was used to measure absorbance at wavelength of 365 nm with spectrophotometer.

2.8.2. Total free proline content

The proline level was quantified through pulverising 0.25 g of freshly harvested leaf material in 5 mL of a 3 % $C_7H_6O_6S$ solution, and subsequently filtering the resulting extract. A volume of 1 mL of the filtrate was kept in a test tube together with 1 mL of $C_6H_4(CO)_2C(OH)_2$ and 1 mL of CH_3COOH. Mixture was then heated in water bath for 90 minutes at temperature of 100 °C. Two distinct layers were formed as a result of the vortexing process. Absorbance of the upper layer, which had a pinkish colour, was determined at wavelength of 520 nm with spectrophotometer.

2.8.3. Anthocyanin

To test the amount of anthocyanin, 0.2 g of crushed leaf sample was combined with 5 mL of CH_3OH that had been acidified. The acidified CH_3OH solution was obtained by combining 120 mL of CH_3OH with 1 mL of hydrochloric acid. Samples were kept in suitably labelled test tubes, which were subsequently moved to water bath at a temperature of 50°C for duration of 1 h. Subsequently, test tube was extracted, and absorbance was measured at wavelength of 535 nm employing a spectrophotometer (Murray and Hackett, 1991).

2.8.4. Total soluble proteins (TSP)

A Bradford reagent produced in order to quantify concentration of TSP. The reagent prepared with combining 1 litre of DI water with 100 millilitres of 85 % H_3PO_4, 0.1 g of brilliant blue, and 50 millilitres of 95 % CH_3CH_2OH. Freshly prepared reagent underwent filtration employing filter-paper, repeating the process 3–4 times. Each test tube was filled with 5 mL of reagent and 0.1 mL of leaf sample. The spectrophotometer was used to measure the absorbance at a wavelength of 595 nm.

2.8.5. Total soluble sugars (TSS)

To determine total amount of soluble sugar (TSS), a sample of 0.5 g fresh leaf material was extracted with a solution of 80 % CH_3CH_2OH. A total of 100 mL of ethanol extract was mixed with 3 mL of enthrone reagent, which had been earlier produced in 72 % sulphuric acid. Subsequently, the concoction was subjected to a temperature of 95 °C for duration of 15 min. The reaction mixture was cooled at ambient temperature for duration of 30 minutes. The spectrophotometer employed to determine absorbance of combination at a wavelength of 620 nm (Yemm and Willis, 1954).

2.8.6. Ascorbic acid (AsA)

Quantity of the endogenous Ascorbic Acid was determined using the procedure proposed by Mukherjee and Choudhuri (1983). To extract the desired substance, a fresh leaf sample weighing 0.20 g was crushed using 5 mL of a solution containing 6 % $C_2HCl_3O_2$. The experiment involved adding 4 mL of extract, 2 mL of a solution containing 2 % $C_6H_6N_4O_4$ in an acidic medium, and a small amount of CH_4N_2S in 70 % CH_3CH_2OH. The solution was subjected to heat in a water bath for duration of 15 minutes, followed by cooling to the ambient temperature. Following the cooling process, 5 mL of a solution containing 80 % Sulfuric Acid was incorporated. The resulting mixture was subsequently kept at a temperature of 0 °C using ice. The spectrophotometer employed to determine absorbance at a wavelength of 530 nm.

2.8.7. Flavonoids

Marinova et al. (2005) conducted measurements to determine the flavonoid levels. After a short period of incubation at a temperature of 25 °C, 1 mL of CH_3CH_2OH extract was mixed to solution comprising 300 L of Sodium nitrate. Subsequently, 300 µL of aluminum chloride was mixed to mixture, which was then placed to incubate at room temperature for duration of 5 minutes. Additionally, 2 mL of NaOH (1 M) was mixed to mixture, and it was let to cool at room temperature for the 10 minutes. Volume of the mixture was augmented to the 10 mL by adding DI water. The spectrophotometer detected an absorbance at a wavelength of 510 nm.

2.8.8. Total phenolics

The total phenolic level was measured with technique stated by Julkenen-Titto (1985). The extraction process involved employing 10 mL of 80 % C_3H_6O to extract 0.5 g of leaf material. 1 mL of the liquid that settled at the top after centrifugation was combined with 5 mL of a solution containing 20 % sodium carbonate and 1 mL of a reagent called Folin-Ciocalteu phenol. DI water was included to increase total volume of mixture to 10 mL. Spectrophotometer employed to determine the absorbance of reaction mixture at a wavelength of 750 nm.

2.9. Ciprofloxacin level in plants

The plant samples, weighing 1 g each, were exposed to digestion in conical flask comprising 10 milliliters of mixture of HNO_3 and $HClO_4$ at a ratio of 3:1 (v/v). The flask was left undisturbed over-night and then heated on a hot-plate. An additional 5 milliliters of nitric acid were added until a transparent solution was achieved (Lillenberg et al., 2010). Plant digests were analyzed using an atomic absorption spectrophotometer to determine the ciprofloxacin concentrations. The concentrations of roots and shoots, as well as the levels of sodium and potassium, were measured using a flame photometer. This was done by dissolving the ashed samples in nitric acid.

2.10. Statistical analysis

The record data was analyzed with one-way ANOVA at a 5 % probability level. The analysis was achieved with SPSS Statistics, Version 28.0. Tukey's HSD post hoc test was applied for making multiple comparisons of means when it was deemed significant. The combined impact of salinity and biochar was examined with Two-way ANOVA. The Pearson correlation coefficients between several variables were calculated with aforementioned statistical tool.(Fig. 1)

3. Results

3.1. Plant growth-related parameters and grain yield

Fig. 2 displays data about plant biomass, growth, as well as grain yield. The spikes and shoots length, and dry biomass of shoots, roots, grains, and spikes exhibited increased trend as the levels of NaCl in the soil increased, as shown in Fig. 1. The spike and shoot lengths of the 40 mM NaCl treatment were increased by 29.7 % and 20.4 % respectively, then the control group. Biochar addition into soil enhanced the lengths of both the shoots and spikes in a dose-additive way, significantly enhanced even under higher stressed conditions. The spike and shoot length had a substantial increase of about 24 and 21 %, respectively, in the nZVI-loaded biochar treatment than control group (Fig. 1 A and B). The dry weights of spikes, shoots, roots, and grains exhibited a substantial increase when the saline levels in the soil increased, as shown in Fig. 2. When exposed to a concentration of 40 mM NaCl, there was an improvement of approximately 20 %, 26 %, 23 %, and 27.3 % in the weights of roots, spikes, shoots, and grains, respectively than control. The administration of nZVI-loaded biochar to the soil resulted in a considerable increase in the dry weights of various parts of plants. The

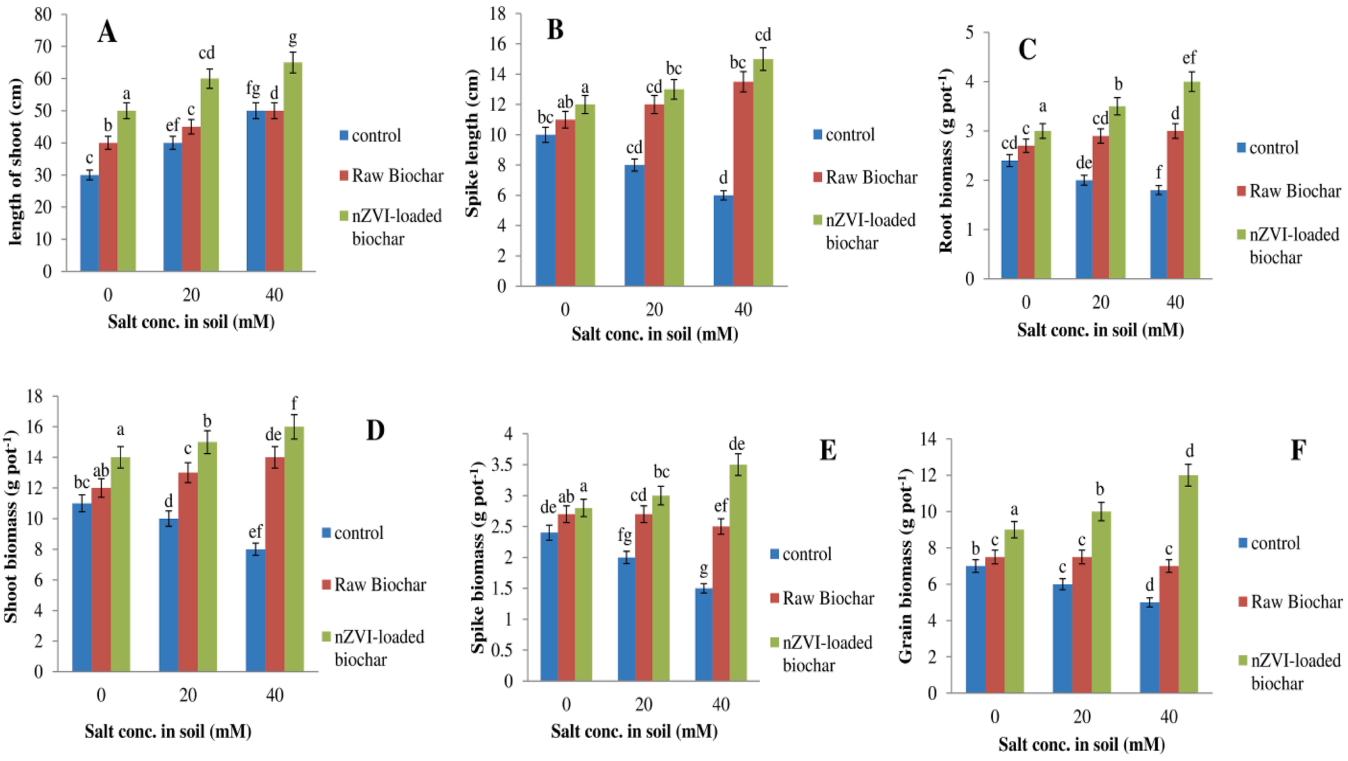

Fig. 1. (A) length of shoot, (B) length of spike, (C) biomass of root (D) biomass of shoot (E) biomass of spike, as well as (F) biomass of grains of wheat cultivated in historically Ciprofloxacin-polluted soil and amended with enhancing doses of salinity and raw and nZVI-loaded biochar. Values are means ± SD ($n = 4$). Diverse lowercase letters specify significant difference among biochar treatments at $p < 0.05$.

most significant increase in the biomass of roots, shoots, spikes, and grains was likewise found when nZVI-loaded biochar was supplied, resulting in an approximate rise of 44 %, 14 %, 29 %, and 26 % than control, respectively. Nevertheless, the addition of nZVI-loaded biochar + 40 mM NaCl did not result in a notable enhancement in root, shoot and grain biomass, when compared to the effects of salt stress alone.

3.2. Parameters related to gas exchange and photosynthetic pigments

The quantities of photosynthetic pigments (Chlorophyll a and b, total Chlorophyll, and carotenoids) in the leaves showed a significant increase after nZVI-loaded biochar treatments, as the saline levels in the soil increased, as seen in Fig. 2A - D. The photosynthetic pigments levels experienced a significant enhancement of around 30 % and 41 % respectively after biochar application, when exposed to a concentration of 40 mM NaCl, as compared to the control group. The administration of nZVI-loaded biochar noticeably enhanced the chlorophyll levels, total Chl, and carotenoids than the control treatment and NaCl treatments without biochar addition, providing the best results in higher salinity levels. The highest levels of Chl a, Chl b was seen in the nZVI-loaded biochar treatment, representing approximately 69 % and 64 % respectively compared to control group. The presence of salt stress resulted in reduction in Pn, Gs, Tr, and WUE than the control group (Fig. 2E–H). The use of raw biochar and nZVI-loaded biochar had a substantial impact on these gas exchange-related parameters than without-biochar treatment. The highest enhancement in WUE, Tr, Pn, and Gs was detected in the treatment with a nZVI-loaded biochar treatment, which exhibited a rise of approximately 95 %, 49 %, 59 %, and 57 % than the control, respectively. The level of intracellular CO_2 is shown in Figure S2.

3.3. Description of EL, and Reactive oxygen species (ROS)

The data related to oxidative stress in leaves, specifically EL, MDA, and Hydrogen peroxide is presented in Fig. 3. The application of NaCl resulted in elevated levels of EL, MDA, and Hydrogen peroxide in the leaves than the control group. The highest rise in EL, MDA, and Hydrogen peroxide levels (about 29 %, 19 %, and 24.6 % respectively) than control was recorded in the treatment with 40 mM NaCl. The utilization of raw biochar and nZVI-loaded biochar resulted in a notable reduction in EL, MDA, and Hydrogen peroxide levels as compared to the NaCl treatment alone, as illustrated in Fig. 3. The nZVI-loaded biochar supply exhibited the least amount of EL, MDA, and Hydrogen peroxide concentration.

3.4. Enzymatic antioxidants activities

The data related to antioxidants activities in leaves, specifically CAT, SOD, and POD, APX and GPX is presented in Fig. 4 The presence of salt stress resulted in a reduction in the activities of SOD and CAT, while it improved the activity of POD, GPX, and APX than control. The use of nZVI-loaded biochar resulted in a decrease in peroxidase (POD) activity and rise in SOD, CAT, APX, and GPX levels compared to NaCl treatments without biochar utilization, as shown in Fig. 4A–E.

3.5. Non-Enzymatic antioxidants activities

The levels of TSS, flavonoids, and TSP showed a significant rise of 43 %, 40 %, and 41 % under ciprofloxacin-contaminated saline conditions after nZVI-loaded biochar was higher than control treatment. The highest growth was observed under (40 mM NaCl) after nZVI-loaded biochar, as shown in Fig. 5. The concentrations of phenolics, ascorbic acid, anthocyanin, proline, and glycine betaine increased by 99 %, 51 %, 39 %, 29 %, and 51 % respectively following the nZVI-loaded biochar application, noticed under (40 mM NaCl), was greater than control group.

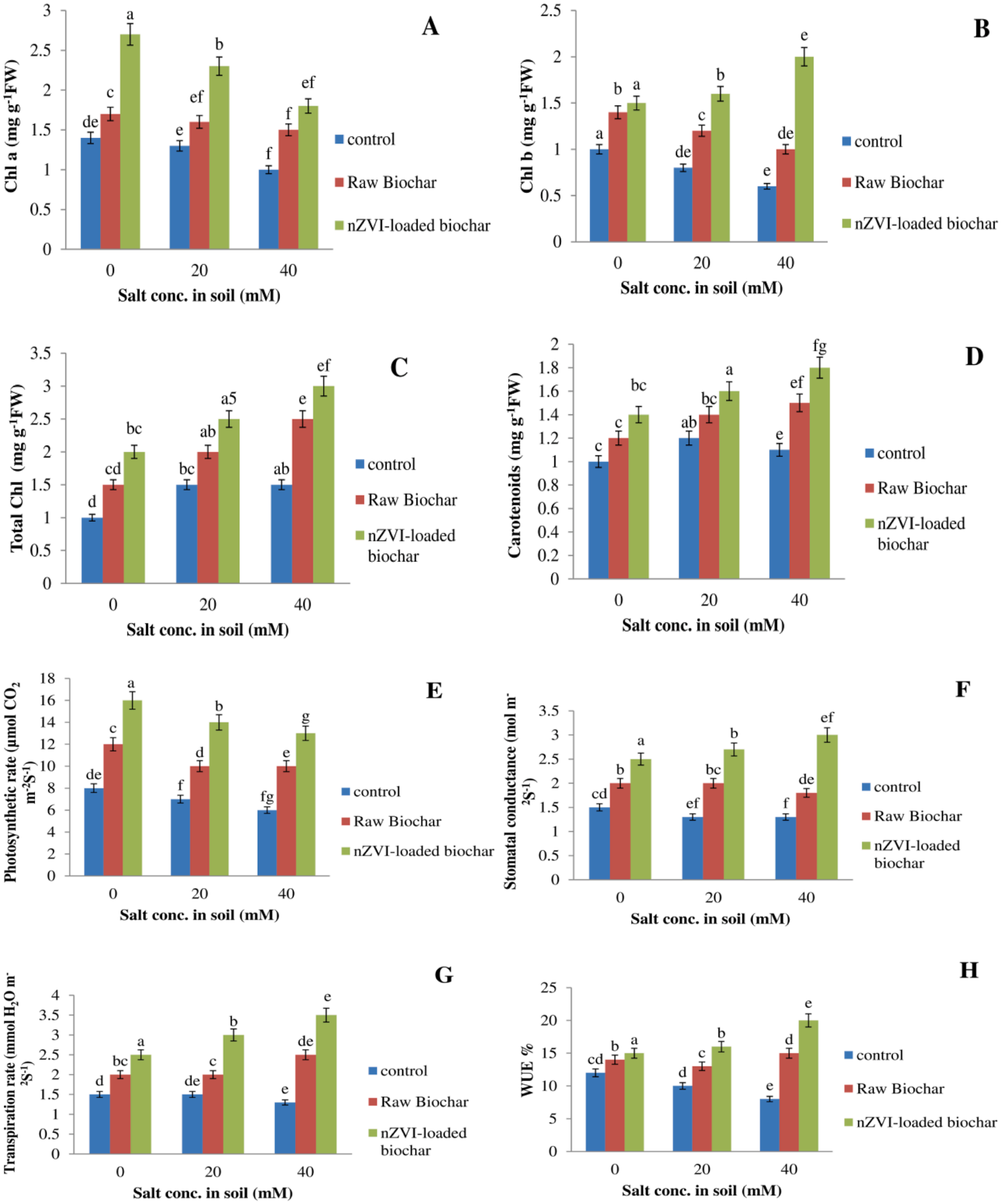

Fig. 2. Photosynthetic pigments (A-D) and gas exchange-related attributes (E–H) of the wheat cultivated in historically Ciprofloxacin-polluted soil and amended with enhancing doses of salinity and raw and nZVI-loaded biochar. Values are means ± SD (n = 4). Diverse lowercase letters specify significant difference among biochar additions at p < 0.05.

3.6. *Ciprofloxacin distribution in plants in plant*

The concentration of ciprofloxacin in roost, shoots, and grains rose as the level of salinity in the soil increased (Fig. 6A–C). The plant components treated with 40 mM NaCl showed the highest rise in ciprofloxacin level, with approximately 30 %, 33 %, and 46 % in the roots shoots, as well as grains, respectively, relative to the control. The addition of nZVI-loaded biochar resulted in significant decrease in the level of ciprofloxacin in these plant sections than treatments where biochar was not applied. The shoots, roots, and grains had the lowest amount of ciprofloxacin in the nZVI-loaded biochar treatment, which was approximately 49 %, 47 %, and 39 % lower than the control, respectively.

3.7. *Soil characteristics and levels of available ciprofloxacin after harvesting*

Table 3 displays the findings of post-harvest soil EC, pH and available ciprofloxacin levels. Salinity elevated soil pH in comparison to control, and the addition of raw and nZVI-loaded biochar further elevated the soil pH in comparison to the corresponding treatments without biochar administration. The salinity had a significant effect on the electrical

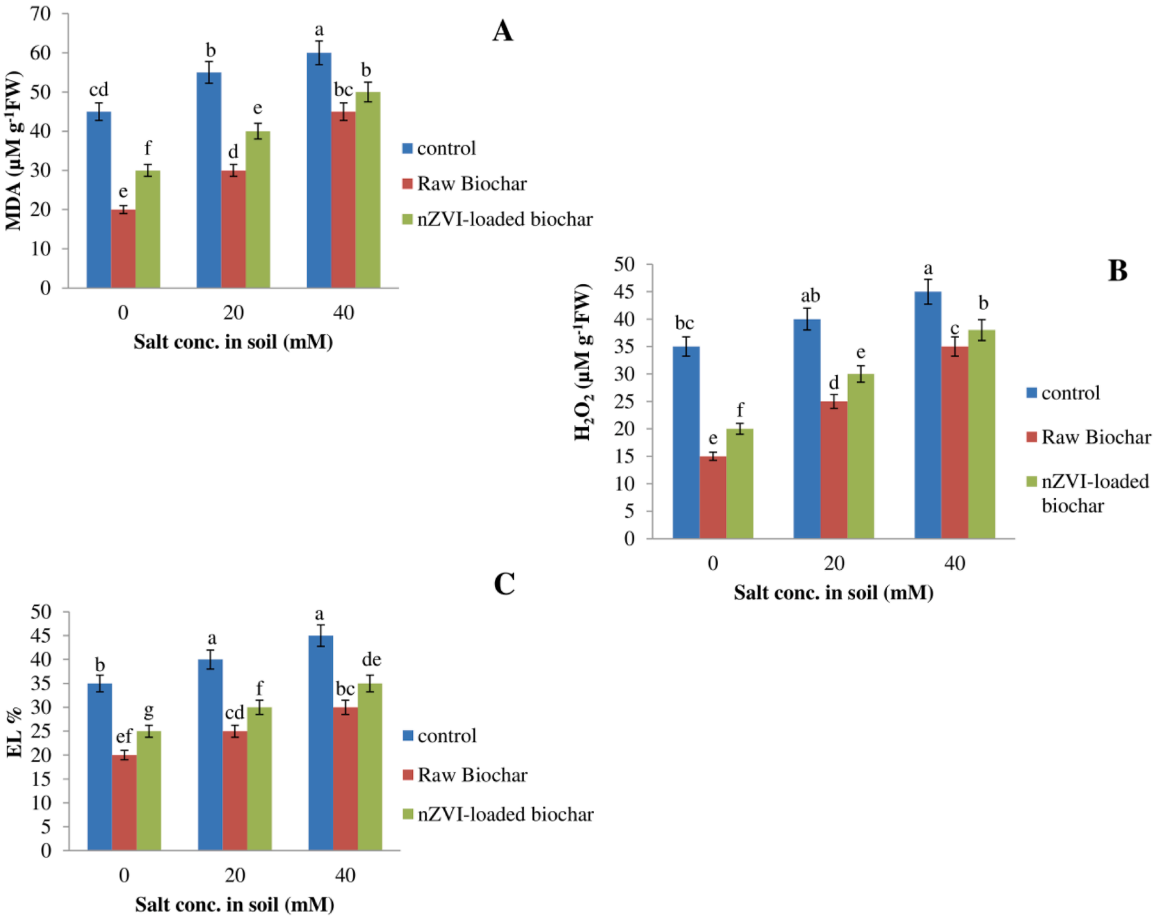

Fig. 3. (A) MDA levels (B) H_2O_2 level (C) EL in leaves of wheat cultivated in a historically Ciprofloxacin-polluted soil and amended with enhancing rates of salinity and raw and nZVI-loaded biochar. Values are means ± SD (n = 4). Diverse lowercase letters specify significant difference among biochar treatments at $p < 0.05$.

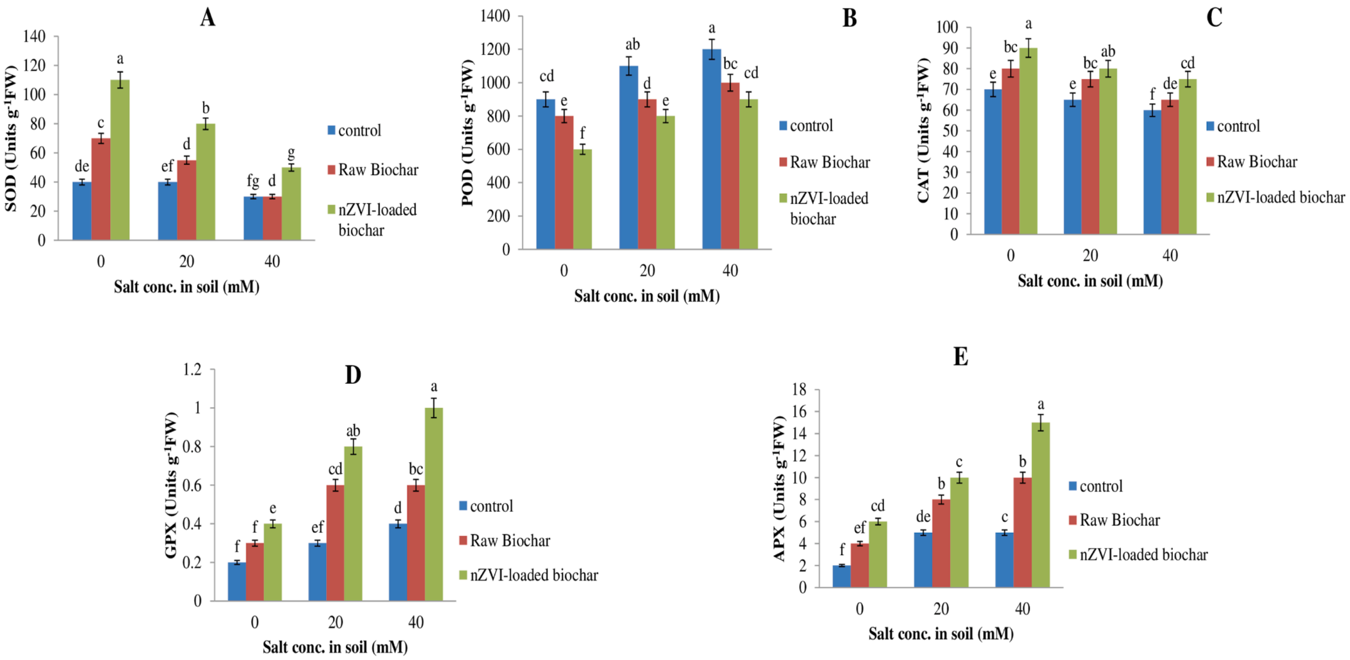

Fig. 4. (A) SOD levels (B) POD levels (C) CAT levels (D) GPX levels (E) APX levels of wheat cultivated in historically Ciprofloxacin-polluted soil and amended with increasing rates of salinity and raw and nZVI-loaded biochar. Values are means ± SD (n = 4). Diverse lowercase letters specify significant difference among biochar treatments at $p < 0.05$.

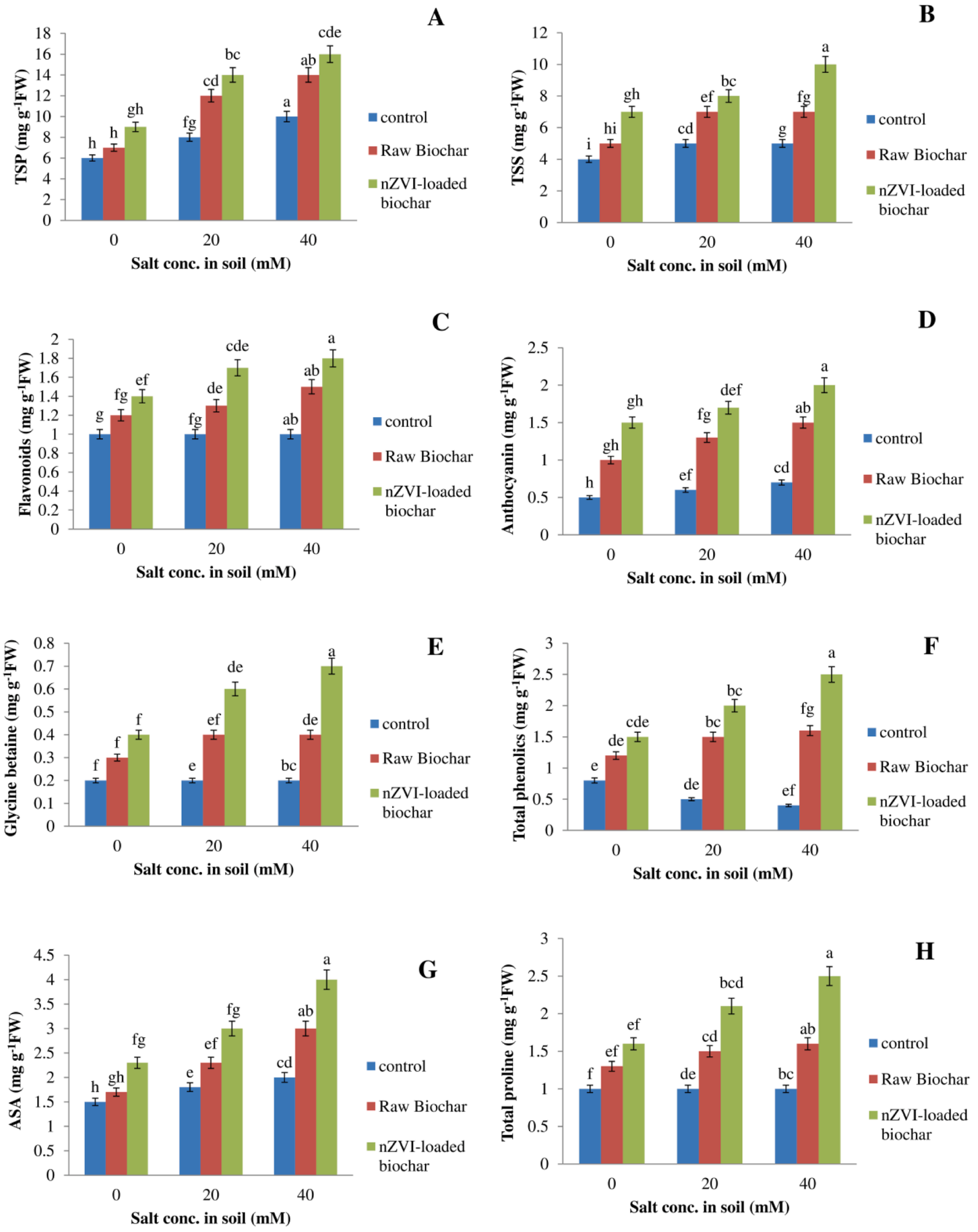

Fig. 5. Non-Enzymatic antioxidants activities levels of wheat cultivated in a historically Ciprofloxacin-polluted soil and amended with increasing rates of salinity and raw and nZVI-loaded biochar. Values are means ± SD (n = 4). Diverse lowercase letters specify significant difference among biochar treatments at $p < 0.05$.

conductivity of the soil when saturated with paste. The nZVI-loaded biochar application significantly elevated the electrical conductivity of the soil in the absence of salt stress, when compared to the control. Nevertheless, the addition of nZVI-loaded biochar resulted in a decrease in soil EC under 20 mM salt stress, as compared to the treatment without nZVI-loaded biochar. While comparing the effects of 40 mM salt stress to the administration of nZVI-loaded biochar + 40 mM NaCl, the soil EC reduced. The salinity caused an increase in the amount of ciprofloxacin in the soil, with the highest amount of bioavailable ciprofloxacin seen at a salt stress level of 40 mM. The addition of nZVI-loaded biochar to the soil led to a considerable reduction in the amount of extractable ciprofloxacin compared to treatments where nZVI-loaded biochar was not applied. The lowest recorded content of ciprofloxacin was found in the treatment with biochar (Table 3).

Values are means ± SD (n = 4). Different lowercase letters specify a significant difference among biochar treatments at $p < 0.05$

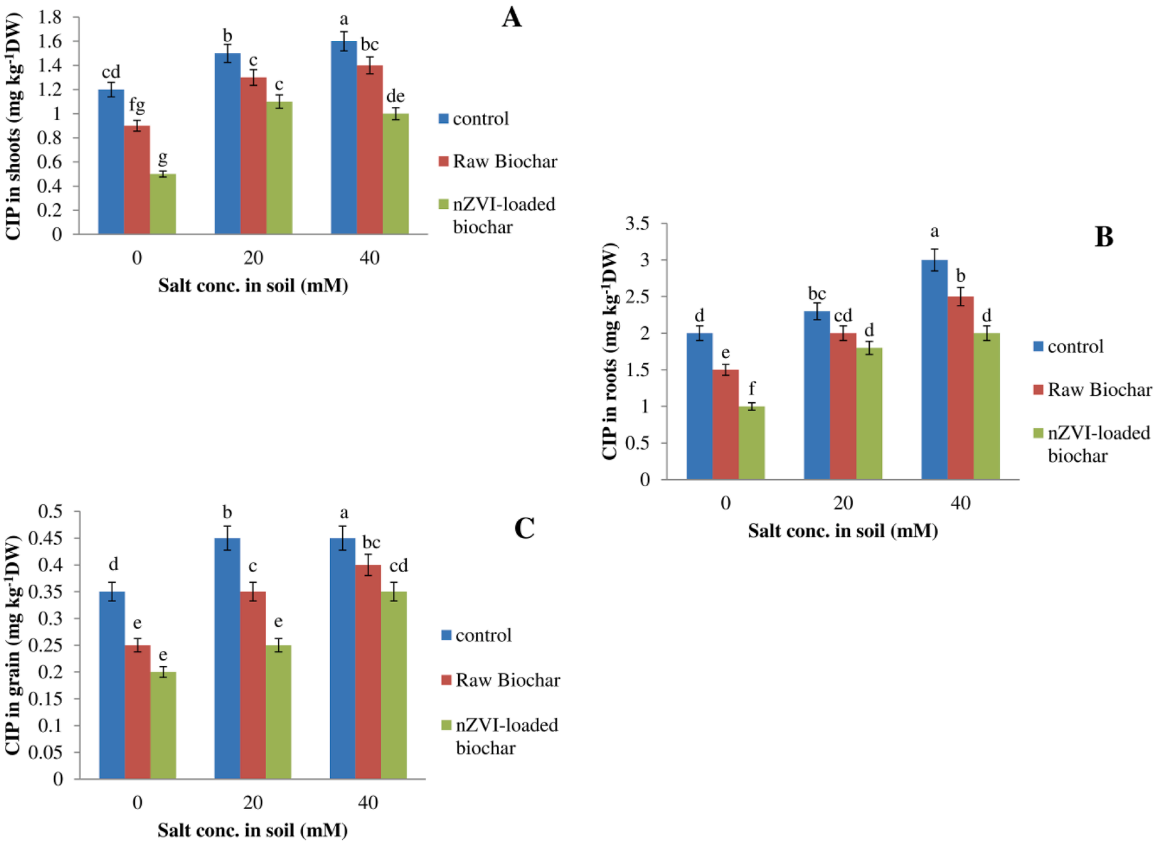

Fig. 6. (A) CIP in shoots (B) CIP in roots (C) CIP in grain of wheat cultivated in a historically Ciprofloxacin-polluted soil and amended with increasing rates of salinity and raw and nZVI-loaded biochar. Values are means ± SD (n = 4). Diverse lowercase letters specify a significant difference among biochar treatments at $p < 0.05$.

Table 3
The extractable concentration of Ciprofloxacin (CIP), electrical conductivity of saturated soil paste (ECe), and soil pH were measured after the harvest of crops in historically Ciprofloxacin-contaminated soil. The soil was planted with wheat plants treated with raw biochar, and nZVI-loaded biochar and subjected to salinity.

Salt level (mM)	Treatments	Soil pHs	ECe (dSm^{-1})	Ciprofloxacin (mg kg^{-1})
0 mM	Control	7.29 ±0.01e	3.19 ±0.03 g	0.40±0.03 cd
	Raw-biochar	7.49 ±0.02c	4.31 ±0.23 f	0.26±0.01e
	nZVI-loaded biochar	7.69 ±0.02ab	4.8±0.39e	0.20±0.02e
20 mM	Control	7.35 ±0.01de	8.39 ±0.22c	0.46±0.04ab
	Raw-biochar	7.50 ±0.02c	7.91 ±0.02d	0.41±0.02bc
	nZVI-loaded biochar	7.64 ±0.03b	7.81 ±0.24d	0.34±0.01d
40 mM	Control	7.39 ±0.01d	11.31 ±0.19b	0.53±0.03a
	Raw-biochar	7.68 ±0.03ab	10.92 ±0.20b	0.43±0.04bc
	nZVI-loaded biochar	7.72 ±0.03a	12.29 ±0.31a	0.37±0.02 cd

4. Discussion

In this study, the biomass and growth of wheat were found to be reduced in the control group (0 % Biochar + 0 mM NaCl). Additionally, the application of salt stress affected the plant biomass and growth, as shown in Fig. 1. Salinity-induced stress induces ionic imbalance, impacting various facets of crop growth, for instance root architecture, photosynthesis processes, and cellular metabolism are disrupted by decreased mineral nutrients uptake under salt-induced stress. The synergistic impact of the combination of Ciprofloxacin and NaCl on plant root length and height exceeded each of the effects of Ciprofloxacin and NaCl treatments. Zhong et al. (2024) reported that the combination of metal, paclobutrazol, and NaCl influenced maize biomass and growth differently across the examined cultivars. In some cases, it resulted in a decrease, whereas in others, it resulted in a rise, in comparison to treatments with Cd or Na alone. The reduction in wheat growth and biomass under salinity stress in the present study may be ascribed to significant structural disturbance of various plant components, including the chloroplast, nucleus, and mitochondria (Zhong et al., 2024). In our study, reduction in the root biomass under salt stress may be attributed to sodium ions accumulation in saline soils (Mahmood et al., 2024). The administration of nZVI-loaded biochar in soil resulted in enhanced plant biomass and growth, except for the treatment involving nZVI-loaded biochar + 40 mM NaCl than without biochar treatments (Fig. 1), it was attributed to the application of biochar also diminished the adverse impacts of salt by binding sodium ions on its exchange site and enhancing the levels of soil potassium ions and moisture (Hameed et al., 2024), enhancement of nutrient absorption, particularly of magnesium and iron, was ascribed to the loading of nZVI onto biochar. Research indicates that the application of biochar enhances plant biomass and growth in potatoes, maize, and wheat when subjected to salt stress (Wang et al., 2021, Cui et al., 2023; Taratima et al., 2023; Rafique et al., 2024), biochar has a greater ability to absorb salt, which can help decrease the uptake of sodium ions and therefore alleviate the negative effects of soil salinity (Hafez et al., 2020). The enhanced nutrient availability and heightened soil enzyme activity resulting from the addition of biochar may have contributed to the increase of plant dry weights. An inverse correlation found between oxidative damage and plant biomass and growth in soils contaminated with contaminants. The results of our study align with the analysis conducted by (Li et al., 2019), indicating that the application of a composite of nZVI and biochar positively impacts plant biomass. Furthermore, the integration of biochar into the soil has been shown to enhance the availability of essential cations and increase the concentrations of total nitrogen and phosphorus.

The presence of both salinity and ciprofloxacin stress led to a decrease in photosynthetic pigments (chlorophyll and carotenoids) levels and gas exchange characteristics. However, the administration of nZVI-loaded biochar significantly enhanced these plant parameters under both stresses alone and combined ciprofloxacin + salinity-induced stress conditions than without ciprofloxacin supply treatments (Fig. 2). The incorporation of raw to the soil increased the levels of chlorophyll a and b, total chlorophyll, and level of carotenoids, by 18.2 %, 22.3 %, 23 %, and 17 %, respectively, compared to the control. Nevertheless, the addition of nZVI-loaded biochar resulted in a 33.4 % rise in chlorophyll a concentration and a 37.8 % increase in chlorophyll *b* content. Both treatments in current study resulted in a greatly increase in the photosynthetic rate. Results indicated that plants treated with raw biochar exhibited a notable 23.1 % enhancement in photosynthesis compared to the control group. The incorporation of nZVI-loaded biochar led to a substantial enhancement of 51.4 % in the photosynthetic rate. In comparison to the control treatment, the incorporation of raw biochar amendment resulted in a 15 % increase in respiration rate, but the incorporation of nZVI-loaded biochar amendment caused a 26.3 % rise in respiration rate. Many plant species including potatoes, wheat, and corn have been found to exhibit an increment in chlorophyll levels, Pn, as well as Gs in the leaves when treated with biochar under salt stress (Murtaza et al., 2024). Both applications also resulted in greatly increase in stomatal conductance. The nZVI-loaded biochar showed the maximum stomatal conductance, measuring 90.3 %. Both biochar including raw and nZVI-loaded biochar showed a comparable pattern in the rise of intercellular CO_2. Of all the treatments administered, the nZVI-loaded biochar exhibited the highest intercellular CO_2 concentration, recorded at 29.6 %. Plants necessitate chlorophyll for food synthesis through photosynthesis. Photosynthesis is more efficient owing to the abundant availability of chlorophyll (Yang et al., 2023; Jin et al., 2022). Plants accumulate contaminants and hinder the absorption of essential minerals required for chlorophyll production. The enhancement of plant defense mechanisms and reduction in oxidative stress may account for the detected increase in chlorophyll levels. Both raw and nZVI-loaded biochar exhibited a linear rise in chlorophyll a and b levels. This indicates that amendments protected chlorophyll against damage caused by ciprofloxacin and salinity. Biochar has the capacity to enhance the absorption of sodium ions in the soil. This process promotes nutrient release and alleviates salt stress by improving water retention capacity and increasing carbon storage. Consequently, there is a significant improvement in photosynthetic activity, stomatal conductance, and transcription rates. Also, addition of raw biochar and nZVI-loaded biochar has reduced the uptake of ciprofloxacin and salinity in the soil. It has also enhanced the levels of chlorophyll and improved photosynthetic characteristics, as reported by Wang et al. (2023) and Zhao et al. (2020).

Oxidative stress impacts several cellular activities in plants through the induction of lipid peroxidation and the impairment of nucleic acids (Sytykiewicz et al., 2019). On the other hand, plants have specific antioxidant enzymes like CAT, POD, and SOD that effectively eliminate ROS generation (Sachdev et al., 2021; Victoria et al., 2023). For instance, SOD converts $O_2\bullet^-$ into H_2O_2, CAT directly changes H_2O_2 into H_2O and O_2, and Peroxidase (POD) catalyzes H_2O_2. ROS detoxification takes place in plants, ensuring a balance between the generation and breakdown of reactive oxygen species for normal plant functioning (Sytykiewicz et al., 2019). Nevertheless, when plants are exposed to stressful conditions, they are unable to effectively eliminate the ROS; resulting in the onset of oxidative-stress in plants (Rezayian et al., 2019). In our research, the addition of raw biochar amendment resulted in a 39.2 % rise in the SOD level and a 23.6 % increase in CAT level, as compared to the control. The findings indicated that the highest increase in the activity of SOD and CAT occurred in plants treated with nZVI-loaded biochar. In comparison to the control, the incorporation of nZVI-loaded biochar amendment led to a 63.1 % enhancement in SOD activity and a 30.7 % augmentation in CAT activity. Following the application of nZVI-loaded biochar, POD activity decreased by 329.7 %. The current research has observed a promotion in EL, H_2O_2, and MDA due to increased salinity levels. Plants under metals or organic contamination stress generate a significant quantity of reactive oxygen species (ROS) in their membranes. The reactive oxygen species (ROS) are oxidized by the fatty acids existent in the membranes. Current findings indicate that plants grown in soils amended with raw biochar and nZVI-loaded biochar demonstrate decreased oxidative stress due to lower levels of CIP and salt. A previous study revealed that biochar had great effect in mitigating oxidative stress in soybean, amaranth, and radish plants cultivated in soils contaminated with different metals (Unsal et al., 2020). The antioxidant enzyme activities of plants indicate their capacity to mitigate the oxidative stress induced by heavy metals and organic pollutants. After to the incorporation of biochar, wheat plants may have encountered reduced oxidative stress as a result of enhanced antioxidant enzyme levels (Hajam et al., 2023; Sachdev et al., 2023). According to our results, under saline conditions exhibited greatly rise in the concentrations of TSS, TSP, and endogenous AsA. Accumulating osmolytes, including TSS, TSP, as well as endogenous AsA, is a key physiological characteristic that indicates tolerance to salt in plants. This process is broadly used by plants to manage salt stress (Ilyas et al., 2024). These chemicals protect cells from salt stress by maintaining the osmotic balance between the cytosol and the vacuole (Guo et al., 2022). nZVI-loaded biochar significantly enhances ASA, TSP, and TSS levels in both salinity-induced stress and normal environments. Biochar improves the potential of crops to withstand high levels of salt by amending the osmotic balance, which aids sustain the firmness of cell membranes and promote enzymes activity involved in osmolyte metabolism. These mechanisms are vital for protecting plant tissues from damage and ensuring uninterrupted growth and development in salinity (Fu and Yang, 2023).

In our research, secondary metabolites, such as flavonoids, anthocyanins, and total phenolics, showed a rise in response to salinity. These compounds are acknowledged for their essential function in alleviating salinity-induced stress, which can lead to oxidative damage to crops (Kumar et al., 2023). Phenolic substances exhibit antioxidant effects through counteracting free lipid-radicals and inhibiting the transformation of hydro-peroxides into free-radicals. The enhancement antioxidant level assists in removal of ROS, which is expected to promote salt resistance (Saleem et al., 2022). The application of nZVI-loaded biochar markedly increased the concentrations of secondary metabolites, such as phenols, flavonoids, and anthocyanins, in both salinity-induced stress and control treatments in the current study. nZVI-loaded biochar acts as a signalling molecule that triggers several defensive mechanisms in crops. One of these techniques entails the sustaining of membrane robustness and the functioning of enzymes. Secondary metabolites aid plants in preventing tissue damage and scavenging damaging ROS. This improves the plant's defence mechanism against the negative effects of salt, enabling it to continue developing and progressing even in hard conditions (Okla et al., 2024).

Our research revealed that under salinity-induced stress, the glycine betaine levels and proline concentration significantly enhanced. The increase in concentration of these solutes and osmoprotectants in response to salt stress validates the wheat's adaptive mechanism to alleviate the detrimental impacts of stress. Proline is key for stabilizing membrane integrity as it interacts with membrane phospholipids, modifying the hydrated layer around biological macro-molecules. This helps protect cellular structures from the detrimental impact of salt stress. Application of raw and modified enhanced the synthesis of proline and glycine betaine in both salinity-induced stress and normal conditions. Proline and glycine betaine possess the potential to eliminate reactive oxygen species (ROS) generation and withstand the effects of salinity-induced stress. Glycine betaine and proline have the ability to help plants protect their tissues by inhibiting the generation of harmful ROS. This enhances the plant's defence mechanism against the detrimental impacts of salinity, enabling constant growth even in challenging and stressful circumstances (Eghlima et al., 2024; Vu et al., 2023).

Salinity resulted with elevated soil pH, electrical conductivity, and extractable ciprofloxacin concentrations post-harvest. The utilisation of nZVI-loaded biochar led to a reduction in ciprofloxacin concentrations and an elevation in soil electrical conductivity and pH values compared to treatments lacking nZVI-loaded biochar supplementation (refer to Table 3). The decrease in extractable ciprofloxacin concentrations in soil attributed to the adsorption of ciprofloxacin onto biochar, and this adsorption mechanism is repetitively irreversible with limited release of ciprofloxacin into the aqueous medium (Murtaza et al., 2023). The addition of contaminants +NaCl to soil resulted in an increase in pH, electrical conductivity, and the level of contaminants (extracted with water) in the rhizospheric soil after harvesting *C. rossii* than control and individual treatments of NaCl and contaminant alone (Abideen et al., 2022). The application of biochar sourced from poultry waste led to a decrease in soil pH in saline soil compared to soil with salinity alone (Krishnamoorthy et al., 2022). The administration of nZVI-loaded biochar resulted in a surge in the soil EC than soil without biochar. This rise was more evident with higher dosages of biochar in the soil, found by Hematimatin et al., (2024). Nevertheless, the current research detected decreased electrical conductivity values when using nZVI-loaded biochar treatments with 20 mM salt stress. The reduction in electrical conductivity values while using nZVI-loaded biochar improvements may be attributed to the sorption of sodium on the nZVI-loaded biochar sites (Hamoud et al., 2024). The leachates from columns treated with biochar had a lower concentration of sodium compared to the columns that were not treated with biochar, as reported by Hamoud et al. (2024).

5. Conclusion

This work involves the integration of the beneficial attributes of nZVI and biochar to generate nZVI-loaded biochar. A quantitative assessment was carried out to measure the potential impacts of raw and nZVI-loaded biochar on the ciprofloxacin and salinity stress in wheat biochemical and physiological response and ciprofloxacin available in plants after post-harvest. The findings of this study demonstrate that the utilization of nZVI-loaded biochar soil is a highly successful approach for promoting the biomass and growth of wheat, as well as mitigating the negative impacts of salinity and ciprofloxacin-induced stress in plants, particularly when exposed to mild salt stress conditions (20 mM NaCl). The utilization of nZVI-loaded biochar resulted in a reduction in oxidative stress and enhancement in antioxidant enzyme level and significantly impacted non-enzymatic activities such as TSS, flavonoids, TSS, phenolics, ascorbic acid, anthocyanin, glycine betaine and proline in presence of both salt and CIP stress. The addition of nZVI-loaded biochar + NaCl resulted in higher chlorophyll levels and improved gas exchange characteristics than NaCl treatments alone. The administration of nZVI-loaded biochar enhanced wheat's resistance to salt and CIP mostly by diminishing the uptake of CIP and sodium generated by NaCl in the roots. The study concludes that nZVI-loaded biochar can rectify the soils dirtied with CIP, and restrict the absorption of these pollutants by wheat plants. Furthermore, nZVI-loaded biochar has the ability to function as a substitute for conventional soil fertilizers, and a novel sustainable approach. Consequently, additional studies will be required to explore the impacts of different rates of nZVI-loaded biochar under simultaneous CIP and salt stress, especially in field trials.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author's contribution

All authors contributed equally to the conception, designing, writing, reviewing, and approval of the final version of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117202.

Data availability

Data will be made available on request.

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