

Zeolite-AMF application enhances wheat productivity, cadmium immobilization, and saline soil health under Cd-contaminated wastewater irrigation

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ABSTRACT

Salinity and heavy metal contamination represent major limitations to sustainable crop production in arid and semi-arid areas, particularly where irrigation relies on low-quality or industrial wastewater. Herein, we evaluated the potential of zeolite and arbuscular mycorrhizal fungi (AMF) to mitigate salinity stress and cadmium (Cd) toxicity in wheat grown under saline soil conditions irrigated with Cd-contaminated wastewater. A split-split plot experiment was conducted with two field capacity (FC) levels (50 and 75%), two AMF treatments (with and without inoculation), and three zeolite application rates (0, 1.2, and 2.4 t ha⁻¹). The integrated application of zeolite and AMF under higher soil moisture significantly improved soil physicochemical properties by reducing pH, electrical conductivity (EC), and exchangeable sodium, while enhancing microbial biomass, enzymatic activities, soil organic matter, and cation exchange capacity. Soil structural quality was noticeably improved, as evidenced by reduced bulk density and augmented porosity and saturated hydraulic conductivity. Notably, zeolite and AMF substantially reduced Cd residues in soil post-harvest and wheat tissues as well, resulting in a pronounced decrease in grain Cd residues. These soil-level improvements were reflected in enhanced plant physiological performance, including improved Na⁺/K⁺ homeostasis, water relations, photosynthetic capacity, and redox homeostasis. Consequently, grain yield and yield components were significantly increased under the combined treatment. Collectively, these findings demonstrate that zeolite-AMF integration under optimized soil moisture establishes an effective, eco-friendly strategy for remediation of saline and Cd-contaminated soils while maintaining crop productivity.

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1. Introduction

Soil salinity has emerged as a major global ecological threat, a pervasive issue affecting agricultural productivity worldwide and ecosystem health (Aryal et al., 2025). Saline-affected soils, characterized by excessive concentrations of dissolved salts within the rhizosphere, disrupt soil structure, impair crop development and nutrient uptake, and lead to reduced crop yields (Zhou et al., 2024). In drought-prone regions, where irrigation is indispensable for sustaining agriculture, the problem is further exacerbated by the use of poor-quality water sources, making it challenging for crops to thrive (Ben Hassen et al., 2025). Addressing this issue requires innovative and sustainable soil management practices to improve soil quality and enhance crop performance under such salt-affected conditions (Paz et al., 2023).

Compounding this issue, the use of cadmium (Cd)-contaminated wastewater for irrigation is a common practice in water-scarce regions, which introduces toxic heavy metals into the soil, further exacerbating soil degradation and food safety, human health, and overall ecosystem stability (Rashid et al., 2023). Cadmium, being both non-essential and highly toxic, can accumulate in soils and plants, creating hazards for crops, food security, and human health (Mushtaq et al., 2025). Cd contamination in soils leads to reduced microbial activity, decreased nutrient availability, and potential uptake by crops (Shah et al., 2023). Its presence in agricultural soils can inhibit plant growth, disrupt physiological processes, and reduce crop yields, ultimately affecting food safety, particularly in already stressed environments such as saline soils over time (Zheng et al., 2024). Therefore, strategies to mitigate Cd accumulation in soils and plants are essential for maintaining sustainable agricultural practices (Navarro-Torre et al., 2023).

Wheat (*Triticum aestivum* L.), a globally important staple crop, provides a primary source of food and essential nutrition (Al-Kofahi et al., 2025). As a primary source of calories and protein for millions of people, maintaining wheat productivity under adverse conditions is critical for food security (Barnes, 2022). Today, it underpins the stability of food security and economic stability (Dangi et al., 2023). Wheat is a highly versatile crop, integral to numerous food products like bread, pasta, and pastries, as well as in industrial applications (Zhang et al., 2023). However, wheat production faces significant challenges, particularly in regions affected by saline soil and heavy metal contamination, often resulting from prolonged irrigation practices (Guo et al., 2025). These adverse conditions can hinder growth, reduce yields, and compromise grain quality, posing threats to both agricultural productivity and food safety (Asgari and Cornelis, 2015). Understanding the impact of saline soil and heavy metal contamination on wheat cultivation is crucial for developing sustainable practices to secure food production in response to environmental and climatic changes, particularly in light of the ever-increasing population, necessitating innovative solutions to mitigate these stresses (Mussarat et al., 2021).

In this context, sustainable soil management practices are urgently needed to address the challenges posed by soil salinity and heavy metal pollution (Anas et al., 2025). Arbuscular mycorrhizal fungi (AMF) and zeolite have gained attention as sustainable and promising soil amendments due to their potential to enhance soil characteristics under abiotic stress (Javaid et al., 2024; Kumar et al., 2025). AMF, beneficial soil microorganisms that establish a symbiotic relationship, enhance plant root function, improving the active absorption of water and nutrients, improve soil structure, and alleviate plant stress by modulating growth, physiological, and biochemical processes (Khan et al., 2025). In saline and heavy metal-polluted soils, AMF can help plants by enhancing root water absorption, increasing antioxidant activity, enhancing soil microbial activity, and reducing the uptake of toxic metals like Cd (Abdallah et al., 2026). Its application is considered a sustainable approach to improving soil quality and potentially enhancing crop resilience in degraded soils (Javaid et al., 2024; Kumar et al., 2025).

Meanwhile, zeolite, a natural aluminosilicate mineral, possesses a high cation-exchange capacity (CEC) and remarkable water-holding

ability (Wang et al., 2025). These properties enable zeolite to retain essential soil nutrients while simultaneously degrading the bioavailability of heavy metals (Mondal et al., 2021). By immobilizing toxic elements and improving soil structure, zeolite enhances soil fertility and supports plant growth, even in challenging environments such as heavy metal-contaminated and saline soils (Javaid et al., 2024). Its application offers a sustainable approach to mitigating soil degradation and promoting agricultural productivity under stress conditions, making it a sustainable solution for enhancing agricultural productivity and soil health under challenging conditions (Feng et al., 2025).

The present research focuses on assessing how the combined application of AMF and zeolite influences soil physicochemical properties, physiological traits, and yield in saline soils treated with wastewater containing cadmium. By exploring the synergistic potential of these amendments on soil properties, plant responses, and crop productivity, this research aims to provide sustainable strategies to improve crop productivity and soil health in challenging environments, ultimately contributing to food security and environmental sustainability.

2. Materials and methods

2.1. Site conditions and soil profile characteristics

The experiment was implemented as an open-field trial at Taawun Village 1, El-Hamool County (31.40° N, 31.17° E), within Kafr El-Sheikh Governorate, Egypt, to examine the functional contribution of arbuscular mycorrhizal fungi (AMF) and zeolite on soil traits and wheat growth in saline soil conditions irrigated with Cd-wastewater, with two field capacities (50% and 75%). A split-split plot arrangement with three replicates was employed. The main plots were allocated to two soil moisture regimes corresponding to 50% and 75% of field capacity. The subplots consisted of two AMF treatments (AMF inoculation [+AMF] and without AMF inoculation [−AMF]), and within the sub-subplots, zeolite was incorporated at three different application rates: 0, 1.2, and 2.4 t ha^{−1}. Baseline soil samples were taken from the top 0–30 cm layer before experimentation to assess physicochemical parameters. The soil was classified as clayey, with initial properties including pH, electrical conductivity (EC), exchangeable sodium percentage (ESP), and organic matter content (OMC), which were 8.29, 5.13 ds m^{−1}, 18.61, and 10.78 g kg^{−1}, whilst heavy metals concentrations such as available Cd, Pb, and Ni contents were 1.09, 4.03, and 2.39 mg kg^{−1}. The field was plowed, harrowed, and leveled before treatment application. Certified seeds of wheat (*Triticum aestivum* L., cv. Sakha 95) were planted on 28 November 2023 at a seeding rate of 120 kg ha^{−1}. Agricultural practices, including fertilization was followed. The crop was irrigated using wastewater, and irrigation scheduling was maintained 50% and 75% of the soil's water-holding capacity.

2.2. Soil preparation and treatments application

2.2.1. Soil preparation

The experimental field was plowed, leveled, and divided into plots of 12 m² (3 × 4 m). Basal doses of fertilizers (33.5% N; 15.5% P₂O₅; 48% K₂O) were applied uniformly across all plots.

2.2.2. Zeolite application

Zeolite (HiMedia Laboratories LLC, Maharashtra, India) was incorporated into the soil at application rates of 0, 1.2, and 2.4 t ha^{−1} and evenly mixed within the upper 20 cm layer before sowing (Aiad et al., 2021). Zeolite (Z) chemical composition was obtained from the National Research Centre, Egypt, was analyzed and the results were as follows: CEC = 155 cmol kg^{−1}, pH = 8.67, EC = 2.03 ds m^{−1}, total C = 8.9%, SiO₂ = 40.76%, TiO₂ = 0.59%, Al₂O₃ = 35.66%, Fe₂O₃ = 0.68%, MnO = 0.01%, MgO = 0.03%, CaO = 0.15%, Na₂O = 0.08%, K₂O = 0.48%, P₂O₅ = 0.19%, loss on Ignition (LOI) = 10.31%, Cd content = 0.01 mg kg^{−1}. The results are expressed as weight percentages

(wt%). The Cd concentration detected in the zeolite was negligible (0.01 mg kg^{-1}), indicating that the amendment itself did not contribute meaningfully to soil Cd loading. Instead, the mineralogical composition and high cation exchange capacity of the zeolite suggest that its primary role was as a soil-conditioning and ion-exchange material, enhancing nutrient retention, ionic buffering, and water-holding capacity, rather than serving as a direct nutrient fertilizer source.

2.2.3. AMF inoculum and application

The AMF inoculum, *Glomus versiforme* (SWERI 112), was applied at a rate of 2.38 kg ha^{-1} (El-maghraby et al., 2024). The AMF inoculum used in this study was sourced from the Soils, Water, and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt. Wheat seeds were surface sterilized by immersing them in a 3% NaClO solution for approximately two minutes, followed by five washes with sterile distilled water containing a few drops of H_2O_2 . The sterilized seeds were first coated with a 1% Arabic gum solution, after which the mycorrhizal inoculum, containing 10^9 spores per gram of dry soil, was introduced. The mixture was left undisturbed for 30 min to allow partial drying and ensure proper adhesion of the inoculum to the seed surfaces. Seeds assigned to the non-mycorrhizal treatment were prepared similarly but without AMF inoculation (–AMF).

2.3. Irrigation management

Water samples from the Kitchener drains, the primary irrigation source used for wheat cultivation, were analyzed before the experiment. The analysis included measurements of pH, EC, sodium adsorption ratio (SAR), and concentrations of Na^+ , Cl^- , SO_4^{2-} , NH_4^+ , Cd, Pb, and Ni. The results were as follows: pH = 7.19; EC = 0.61 dS m^{-1} ; SAR = 1.49; Na^+ = 2.01 mg L^{-1} ; Cl^- = 3.39 mg L^{-1} ; SO_4^{2-} = 0.11 mg L^{-1} ; NH_4^+ = 1.14 mg L^{-1} ; Cd = 0.08 mg L^{-1} ; Pb = 0.24 mg L^{-1} ; and Ni = 0.13 mg L^{-1} . Based on these results, the experimental soil was classified as Cd-polluted, as the cadmium concentration (0.08 mg L^{-1}) exceeded the acceptable irrigation water limit of 0.01 mg L^{-1} . During the investigation, irrigation was applied to maintain two field capacity (FC) levels: 50% and 75% of the soil's water-holding capacity. Soil moisture was monitored regularly to ensure precise maintenance of the desired FC levels. Field capacity levels were maintained gravimetrically throughout the experiment. Soil moisture content was monitored every two days by periodic soil sampling and gravimetric determination relative to previously established field capacity values. Irrigation volumes were adjusted accordingly to maintain soil moisture at either 50% or 75% of field capacity. Based on the seasonal irrigation volumes applied during the experiment, the estimated cumulative Cd input through irrigation water was approximately 320 and 480 g Cd ha^{-1} under the 50% ($\sim 3000 \text{ m}^3 \text{ ha}^{-1}$) and 75% FC ($\sim 4500 \text{ m}^3 \text{ ha}^{-1}$) treatments, respectively. Throughout the growing season, all irrigation events were conducted using wastewater collected from the Kitchener drains, which served as the sole irrigation source during the experiment.

2.4. Soil chemistry

Unless otherwise stated, all chemicals and reagents used in the following assays were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA), HiMedia Laboratories LLC (Maharashtra, India), and Thermo Fisher Scientific (Waltham, MA, USA).

2.4.1. Soil pH and Electrical Conductivity (EC)

Collect five post-harvest samples from the 0–15 cm layer, homogenize, and sieve ($<2 \text{ mm}$). Following standard soil analysis methods, soil pH and electrical conductivity (EC) were measured in a 1:2.5 soil-to-water mixture (Hesse, 1971; Jackson, 1958). The mixture was stirred for 30 min, allowed to settle, and Soil pH and EC were quantified using a digital pH meter and a conductivity meter, respectively.

2.4.2. Percentage of Exchangeable Sodium (ESP)

The exchangeable sodium percentage (ESP) was quantified following the Sodium Adsorption Ratio (SAR). ESP was calculated as described by Seilsepour et al. (2009) using the following equation:

$$\text{ESP} = 1.95 + 1.03 \times \text{SAR} \quad (R^2 = 0.92)$$

This equation implies that ESP is highly correlated with SAR ($R^2 = 0.92$), meaning SAR can be used as a reliable predictor of ESP.

$$\text{whereas SAR} = [\text{Na}^+] / \sqrt{\frac{([\text{Ca}^{2+}] + [\text{Mg}^{2+}])}{2}}$$

Na^+ , Ca^{2+} , and Mg^{2+} were quantified in meq L^{-1} based on the methodology of (Richards, 1954).

2.4.3. CO_2 basal respiration

Basal respiration was measured following Anderson's method (Anderson, 1982). Briefly, 50 g of soil was incubated at 60% of its water-holding capacity in sealed jars at 25°C for seven days. Evolved CO_2 was trapped in NaOH (0.1 M) and quantified via titration with HCl (0.1 M). The cumulative CO_2 evolution was determined over the incubation period as $\text{mg CO}_2 \text{ 100 g}^{-1} \text{ soil day}^{-1}$.

2.4.4. Microbial Biomass Carbon (MBC) and Microbial Biomass Nitrogen (MBN)

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were assessed using the chloroform fumigation-extraction technique according to (Setia et al., 2012). After fumigating soil samples with ethanol-free chloroform for 24 h, extraction was performed with K_2SO_4 (0.5 M). Organic carbon and total nitrogen in the extracts were analyzed using the dichromate oxidation and Kjeldahl digestion methods, respectively. MBC and MBN values were derived using conversion factors of 0.45 and 0.54, respectively, with units of mg kg^{-1} .

2.4.5. Soil Organic Matter (SOM)

The concentration of soil organic matter (SOM) was measured by applying the Walkley-Black wet oxidation technique (Klute and Page, 1982). The soil was oxidized using a mixture of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$; 1 N) and sulfuric acid (H_2SO_4 ; 95%), and the released organic carbon was determined by titration with ferrous ammonium sulfate.

2.4.6. Available Soil Water

Plant-available soil water was assessed by evaluating FC and permanent wilting point (PWP) using pressure plate analysis and subtracting PWP from FC according to (Silva et al., 2014).

2.4.7. Cation Exchange Capacity (CEC)

Cation exchange capacity (CEC) was determined by the ammonium acetate technique (Sumner and Miller, 2018). Soil was saturated with 1 M ammonium acetate (pH 7.0), and the displaced ammonium was quantified via steam distillation and titration with standard acid.

2.4.8. Potassium (K) Content

Soil K availability was determined by ammonium acetate (1 M) extraction, followed by measurement with a flame photometer, as mentioned by (Sparks et al., 2020).

2.5. Soil physical properties

2.5.1. Soil bulk density (BD)

Soil bulk density was measured following the core sampling technique outlined by (Blake and Hartge, 2018). Undisturbed cores (100 cm^3) were obtained using a stainless-steel cylinder. The samples were dried in an oven at 105°C for 24 h, after which bulk density was computed using the standard equation.:

$$\text{BD} = \frac{\text{Oven} - \text{dry soil weight (g)}}{\text{Core volume (cm}^3\text{)}}$$

2.5.2. Total Porosity (TP)

Total porosity was estimated due to bulk density (BD) and particle density (PD), assuming a standard particle density of 2.65 g/cm^3 (Klute, 1986). The calculation was performed using the following formula:

$$\text{Total Porosity (TP; \%)} = \left(1 - \frac{\text{Bulk density}}{\text{Particle density}} \right) \times 100$$

2.5.3. Hydraulic Conductivity (HC)

Saturated hydraulic conductivity (Ks) was obtained by the constant head technique for sandy and loamy soils and the falling head method for finer-textured soils (Klute, 1986). In the constant head method, water was allowed to flow through a saturated soil column under a steady-state condition, and Ks was calculated using Darcy's equation:

$$K_s = \left(\frac{QL}{A\Delta Ht} \right)$$

where:

- Ks = saturated hydraulic conductivity (cm/day)
- Q = volume of water collected (cm^3)
- L = length of the soil column (cm)
- A = cross-sectional area of the column (cm^2)
- ΔHt = hydraulic head difference (cm)
- t = time (day)

2.6. Soil biochemical processes analysis

2.6.1. Catalase activity

Catalase activity was determined using hydrogen peroxide (H_2O_2) decomposition following the Burns' method (Burns, 1982). Succinctly, 5 g of air-dried soil was incubated with 10 mL of 0.3% H_2O_2 solution. The reaction was stopped after 20 min, and the residual H_2O_2 was titrated with KMnO_4 . Values are shown as $\text{mL O}_2 \text{ g}^{-1} \text{ soil hr}^{-1}$.

2.6.2. Invertase activity

Invertase activity was measured using the 3,5-dinitrosalicylic acid (DNS) method (Guan et al., 1986). Soil samples (5 g) were treated with 15 mL of 8% sucrose and 0.1 M phosphate buffer (pH 5.5) and incubated at 37°C for 24 h. The amount of reducing sugar released was determined spectrophotometrically at 540 nm. Results were shown as $\text{mg glucose g}^{-1} \text{ soil hr}^{-1}$.

2.6.3. Phosphatase activity

Phosphatase activity was measured using the p-nitrophenyl phosphate (PNP) method (Schneider et al., 2000). Soil (1 g) was treated with 4 mL of modified universal buffer (pH 6.5) and 0.25 mL of 0.115 M p-nitrophenyl phosphate and incubated at 37°C for 60 min. The reaction was stopped using 0.5 M NaOH, and PNP release was determined spectrophotometrically at 410 nm. Data are shown as $\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$.

2.6.4. Urease activity

Urease activity was determined by measuring ammonia (NH_3) release from urea hydrolysis, following the standard methods of soil enzymology (Dick, 2011). 5 g of soil was incubated with 1.5 mL of 0.08 M urea solution in citrate buffer (pH 6.7) at 37°C for 2 h. The obtained NH_4^+ was extracted with 2 M KCl and measured colorimetrically at 690 nm by a spectrophotometer. Data were shown as $\mu\text{g NH}_4^+\text{-N g}^{-1} \text{ soil hr}^{-1}$.

2.7. Determination of Cadmium residue in soil and plant tissues

2.7.1. Determination of Cd residue in Soil Post-Harvest

Air-dried soil (0.5 g) was ground to a fine powder and sifted through a 0.2 mm strainer. The sample was digested in a 1:1 mixture of HNO_3

and HCl using a microwave digestion system at 120°C until complete breakdown. Once cooled, the digest was filtered through Whatman No. 42 paper and brought to a final volume of 50 mL with deionized water. Cadmium was extracted with EDTA and determined by atomic absorption spectrophotometry (AAS, PerkinElmer 3300, USA) following the procedure described by (Hesse, 1971; Jackson, 1967, 1958).

2.7.2. Determination of Cd Residue in Different Plant Parts

After harvest, five plant samples (roots, shoots, and grains; $\mu\text{g g}^{-1}$) were gently rinsed with deionized water to eliminate surface impurities. Oven drying at 70°C was performed until a consistent mass was obtained. The dried plant material was finely ground using a stainless-steel mill and passed through a 0.5 mm sieve for uniformity. Wet acid digestion with HNO_3 and HClO_4 was employed to extract Cd (Hesse, 1971; Jackson, 1967, 1958). A 0.5 g portion of the dried sample was transferred into a digestion tube, followed by the addition of 10 mL of concentrated HNO_3 . The mixture was allowed to pre-digest overnight at room temperature. Complete digestion was achieved by adding 2 mL of HClO_4 and heating the sample at $120\text{--}150^\circ\text{C}$ until a clear solution formed. Upon cooling, the digest was filtered through Whatman No. 42 paper and brought to a final volume of 50 mL with distilled water. Cadmium was measured at a wavelength of 228.8 nm using atomic absorption spectrophotometry (AAS, PerkinElmer 3300, USA) following the method described by (Cui et al., 2007).

2.8. Measurement of Physiological and Biochemical Responses

2.8.1. Ion homeostasis (Na^+ and K^+ ; $\text{mg g}^{-1} \text{ DW}$)

Dried leaf samples ($n = 5$) were finely powdered, and 0.5 g of each sample was digested in 10 mL of a 3:1 (v/v) $\text{HNO}_3\text{--HClO}_4$ mixture at $120\text{--}150^\circ\text{C}$ until complete clarification (Wolf, 1982). Atomic absorption spectrophotometry (AAS, PerkinElmer 3300) was employed to quantify Na^+ and K^+ , with values expressed as $\text{mg g}^{-1} \text{ DW}$.

2.8.2. SPAD (Chlorophyll Content Index)

SPAD readings were obtained with a Minolta SPAD-502 chlorophyll meter from the middle portion of the third fully expanded leaf of each plant (Ling et al., 2011), averaging measurements from five points per leaf.

2.8.3. Relative Water Content (RWC%)

Leaf samples ($n = 5$) were immediately weighed to obtain fresh weight (FW), floated in distilled water at 4°C for 4 h, blotted, and weighed for turgid weight (TW). After oven-drying at 70°C for 48 h, dry weight (DW) was measured. RWC was determined according to (Barrs and Weatherley, 1962) by the following equation:

$$\text{Relative Water Content (RWC; \%)} = \left(\frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \right) \times 100$$

2.8.4. Stomatal Conductance ($\text{gs, mmol m}^{-2} \text{ s}^{-1}$)

Stomatal Conductance was measured using a portable leaf porometer (AP4, Delta-T Devices, UK) on the abaxial leaf surface at 9:00 AM and 11:00 AM (Liu et al., 2014). Three readings were taken per plant, and the average was recorded.

2.8.5. Electrolyte Leakage

Circular leaf segments (0.5 cm diameter) were submerged in 10 mL of distilled water and kept at 25°C for 24 h. The initial conductivity of the solution (EC_1) was recorded. Thereafter, the samples were subjected to boiling at 100°C for 15 min to achieve total membrane disruption, cooled to ambient temperature, and the final conductivity (EC_2) was measured. Electrolyte leakage percentage was determined following the method of (Bajji et al., 2002):

$$\text{Electrolyte Leakage (El; \%)} = \left(\frac{\text{Initial conductivity (EC1)}}{\text{Final conductivity (EC2)}} \right) \times 100$$

2.8.6. Malondialdehyde content

A 0.5 g portion of fresh leaves was macerated in 5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 9500 $\times g$ for 10 min using Eppendorf 5415 Refrigerated Centrifuge (Eppendorf, Hamburg, Germany). From the supernatant, 1 mL was transferred to a tube containing 4 mL of 0.5% thiobarbituric acid in 20% TCA. The mixture was heated at 95°C for 30 min to allow color development, then cooled and centrifuged. Absorbance was determined at 532 nm with correction at 600 nm, and results were calculated as described in the referenced method (Du and Bramlage, 1992):

Malondialdehyde content (MDA; $\mu\text{mol g}^{-1}$ FW)

$$= \left(\frac{A_{532} - A_{600} \times V}{\text{Sample weight} \times 155} \right)$$

2.8.7. Hydrogen Peroxide content

A 0.5 g sample of fresh leaf tissue was extracted with 5 mL of 0.1% TCA and centrifuged at 13500 $\times g$ for 15 min to obtain a clear supernatant. Subsequently, 0.5 mL of the extract was reacted with 0.5 mL of potassium phosphate buffer (10 mM, pH 7.0) and 1 mL of 1 M KI. The optical density was determined at 390 nm, and the hydrogen peroxide concentration was estimated based on a previously prepared standard curve (Velikova et al., 2000).

2.9. Quantification of Key Antioxidative Enzymes

2.9.1. Ascorbate Peroxidase (APX) Activity

Approximately 0.5 g of fresh leaf tissue was extracted in 5 mL of 50 mM potassium phosphate buffer (pH 7.0) supplemented with 1 mM EDTA and 1 mM ascorbic acid. The homogenate was centrifuged at 13500 $\times g$ for 15 min at 4°C, and the resulting supernatant was collected for enzymatic analysis. Ascorbate peroxidase (APX) activity was determined by following the decline in absorbance at 290 nm, corresponding to ascorbate oxidation in the presence of H_2O_2 . Enzyme activity was calculated using the extinction coefficient of 2.8 $\text{mM}^{-1} \text{cm}^{-1}$ for ascorbate (Jebara et al., 2005) and expressed on a protein basis as $\mu\text{mol mg}^{-1} \text{min}^{-1}$.

2.9.2. Dehydroascorbate Reductase (DHAR) activity

Approximately 0.5 g of fresh leaf material was extracted in 5 mL of 50 mM Tris-HCl buffer (pH 7.5) supplemented with 1 mM EDTA and 1 mM reduced glutathione (GSH). The homogenate was centrifuged at 13500 $\times g$ for 15 min at 4°C, and the clarified supernatant was used as the enzyme source. Dehydroascorbate reductase (DHAR) activity was determined spectrophotometrically by recording the increase in absorbance at 265 nm, reflecting the conversion of dehydroascorbate to ascorbate. Enzyme activity was calculated using a molar extinction coefficient of 14 $\text{mM}^{-1} \text{cm}^{-1}$ for ascorbate (Dorigan de Matos Furlanetto et al., 2021) and expressed on a protein basis as $\mu\text{mol mg}^{-1} \text{min}^{-1}$.

2.9.3. Glutathione Reductase (GR) activity

Leaf samples (0.5 g) were homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.5) supplemented with 1 mM EDTA and 1 mM DTT. The homogenate was centrifuged at 13500 $\times g$ for 15 min at 4°C, and the clarified extract was used for glutathione reductase (GR) assay. Enzyme activity was determined by monitoring the decrease in absorbance at 340 nm resulting from NADPH oxidation. Calculations were performed using the molar extinction coefficient of 6.22 $\text{mM}^{-1} \text{cm}^{-1}$ for NADPH (Lin et al., 2018) and expressed on a protein basis as $\mu\text{mol mg}^{-1} \text{min}^{-1}$.

2.10. Field productivity analysis

Upon reaching physiological maturity, ten plants were randomly chosen from every plot for the evaluation of yield traits, namely grains per spike and thousand-grain mass (g). Grain yield was estimated from a centrally located 4 m^2 quadrat within each plot and subsequently corrected to 14% grain moisture.

2.11. Statistical analysis

All experiments were carried out by a split-split plot design with two field capacity (FC) levels (50% and 75%) assigned to the main plots, two arbuscular mycorrhizal fungi (AMF) treatments (with and without inoculation) allocated to subplots, and three zeolite application rates (0, 1.2, and 2.4 t ha^{-1}) arranged in sub-subplots. Throughout the experiment, the untreated stressed treatment consisting of 50% FC, without AMF inoculation, and without zeolite application (0 t ha^{-1}) was considered the control treatment and used as the reference baseline for all statistical comparisons. All treatments were conducted using three independent biological replicates. All measured soil, physiological, biochemical, and yield variables were exposed to three-way analysis of variance (ANOVA) to evaluate the main effects of FC ($p_{\text{FC}} \leq 0.05$), AMF ($p_{\text{AMF}} \leq 0.05$), and zeolite ($p_{\text{Zeolite}} \leq 0.05$), as well as their two-way interactions ($p_{\text{FC} \times \text{AMF}} \leq 0.05$, $p_{\text{FC} \times \text{Zeolite}} \leq 0.05$, and $p_{\text{AMF} \times \text{Zeolite}} \leq 0.05$) and three-way interaction effects ($p_{\text{FC} \times \text{AMF} \times \text{Zeolite}} \leq 0.05$). ANOVA was used to assess the main and interactive influence of FC, AMF, and zeolite application on soil physicochemical traits, plant physiological traits, and yield components, followed by Tukey's Honestly Significant Difference (HSD) as a post-hoc test to compare all possible pairs of group means.

3. Results

3.1. Effect on physicochemical and biological traits of treated soil

The physicochemical and biological traits of saline soil were significantly improved by increasing soil moisture, AMF inoculation, and zeolite application, with the combined treatment consistently producing the most favorable conditions and the strongest remediation effect (Fig. 1 and Table S1). Soil pH and electrical conductivity (EC) declined progressively with the addition of zeolite and AMF, indicating partial neutralization of alkalinity and effective dilution or immobilization of soluble salts. Briefly, soil pH declined significantly with zeolite and AMF, reaching its lowest value under 75% FC + AMF + 2.4 t ha^{-1} (7.55 ± 0.06), compared with the untreated stressed treatment (50% FC, -AMF, 0 zeolite) (8.21 ± 0.03) (Fig. 1A). Likewise, EC decreased substantially, from $5.20 \pm 0.03 \text{ dS m}^{-1}$ in the non-amended stressed soil to $4.51 \pm 0.04 \text{ dS m}^{-1}$ under 75% FC + AMF + 2.4 t ha^{-1} (Fig. 1B), reflecting effective salinity mitigation. These changes were particularly evident at 75% field capacity, suggesting that adequate soil moisture enhanced ion exchange processes and facilitated salt displacement from the root zone.

Exchangeable sodium percentage (ESP) followed the same trend and was also markedly reduced by zeolite, especially when combined with AMF. The minimum ESP was observed under 75% FC + AMF + 2.4 t ha^{-1} ($16.69 \pm 0.10\%$) compared to $18.87 \pm 0.05\%$ in the untreated stressed treatment (50% FC, -AMF, 0 zeolite) (Fig. 1C), reflecting the high affinity of zeolite for Na^+ and its capacity to replace it with Ca^{2+} and other beneficial cations. On the other hand, basal soil respiration increased substantially under the combined treatment, peaking at $28.48 \pm 0.64 \text{ mg CO}_2 \text{ kg}^{-1} \text{ soil day}^{-1}$ under the best treatment, almost doubling the value recorded in the Cd-stressed non-amended soil ($14.86 \pm 0.33 \text{ mg CO}_2 \text{ kg}^{-1} \text{ soil day}^{-1}$; Fig. 1D), confirming enhanced microbial activity and carbon mineralization. Collectively, these results demonstrate that zeolite and AMF acted synergistically to mitigate salinity constraints and re-establish a more biologically active and

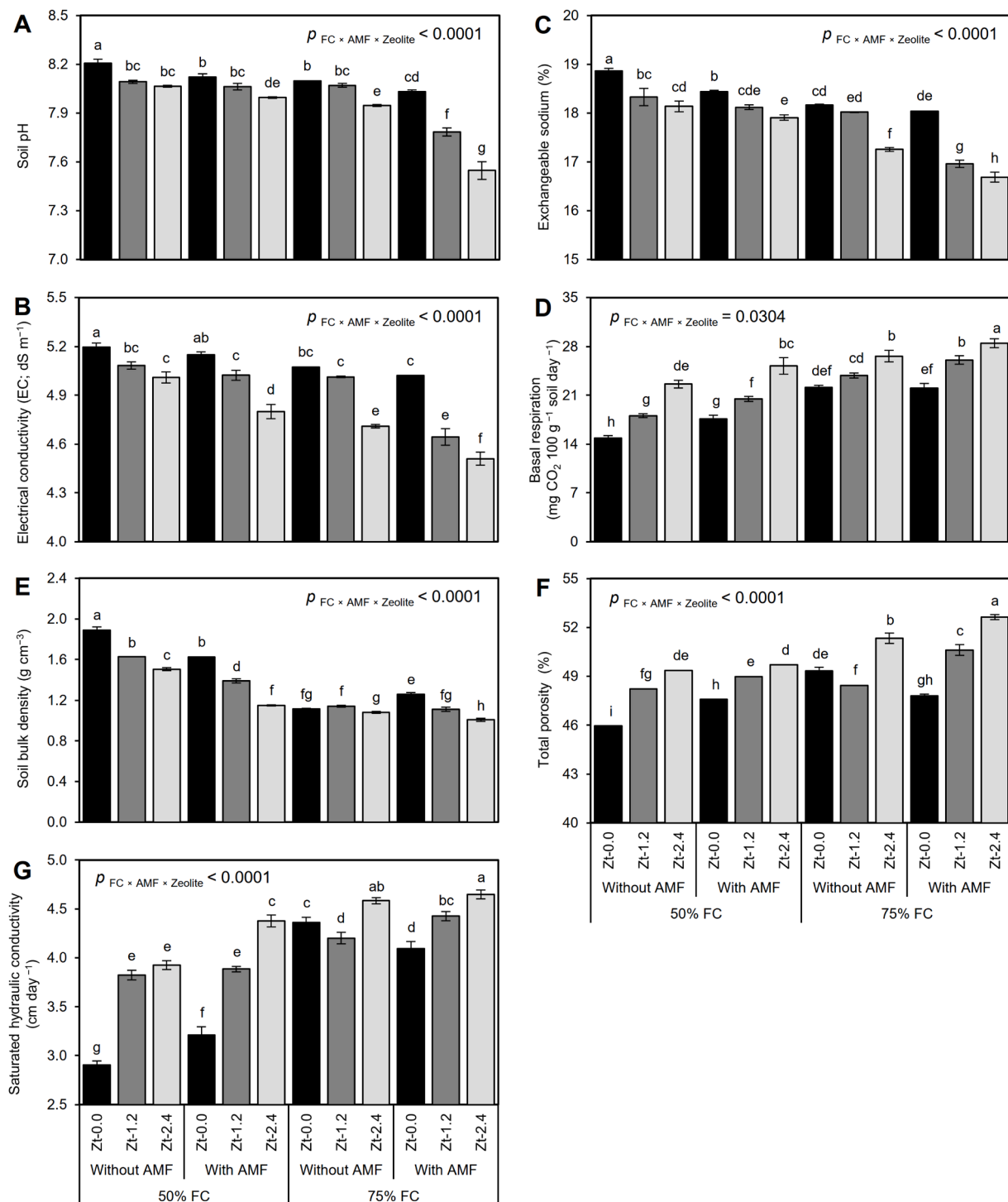


Fig. 1. Effect of field capacity (FC), arbuscular mycorrhizal fungi (AMF), and zeolite on key physicochemical, biological, structural, and physical traits of saline soil cultivated with wheat and irrigated with cadmium-contaminated wastewater. (A) Soil pH, (B) Electrical conductivity (EC; $dS\ m^{-1}$), (C) Exchangeable sodium (%), (D) Basal respiration ($mg\ CO_2\ 100\ g^{-1}\ soil\ day^{-1}$), (E) Soil bulk density ($g\ cm^{-3}$), (F) Total porosity (%), and (G) Saturated hydraulic conductivity ($cm\ day^{-1}$). The experiment was arranged in a split-split plot design with two FC levels (50% and 75%) as main plots, two AMF treatments (with and without inoculation) as subplots, and three zeolite application rates (0, 1.2, and $2.4\ t\ ha^{-1}$) as sub-subplots under saline soil conditions irrigated with cadmium-contaminated wastewater. Bars present means $\pm$ standard deviation (SD) of three independent biological replicates. Different letters denote statistically significant differences among treatment means according to the three-way interaction ($p_{FC \times AMF \times Zeolite} \leq 0.05$), while the same letters indicate no statistically significant differences.

chemically balanced soil environment.

3.2. Effect on structural and physical indicators of saline soil

Soil physical structure quality was significantly improved by zeolite

and AMF, particularly under the higher moisture regime (Table S2). Bulk density declined progressively with increasing zeolite rate and dropped from $1.89 \pm 0.03\ g\ cm^{-3}$ in the non-treated stressed soils to $1.01 \pm 0.02\ g\ cm^{-3}$ under 75% FC + AMF + $2.4\ t\ ha^{-1}$ (Fig. 1E), reflecting reduced soil compaction and improved aggregation.

Correspondingly, total porosity percentage was increased from $45.96 \pm 0.01\%$ in the untreated stressed treatment (50% FC, -AMF, 0 zeolite) to $52.63 \pm 0.16\%$ under 75% FC + AMF + 2.4 t ha^{-1} (Fig. 1F). This effect was further strengthened by AMF inoculation, which likely contributed to aggregate stabilization through hyphal networks and glomalin production. In parallel, saturated hydraulic conductivity (K_s) was also markedly improved, reaching $4.65 \pm 0.05 \text{ cm day}^{-1}$ under the best treatment compared with only $2.90 \pm 0.04 \text{ cm day}^{-1}$ in the untreated soil (Fig. 1G), indicating enhanced pore connectivity and improved water transmission through the soil profile. The observed structural recovery suggests that zeolite and AMF did not merely alter soil chemistry but fundamentally reshaped soil physical architecture, creating a more resilient and functional soil matrix under saline irrigation conditions.

3.3. Impact on biochemical and fertility properties of saline soil

Soil biochemical fertility was strongly enhanced by the combined application of zeolite and AMF, with higher field capacity further

amplifying these effects (Table S3). Microbial biomass carbon (MBC) was increased markedly in amended soils and elevated from only $2.35 \pm 0.06 \text{ mg g}^{-1}$ in the untreated stressed soil to $4.88 \pm 0.10 \text{ mg g}^{-1}$ under 75% FC + AMF + 2.4 t ha^{-1} , representing a 108% enhancement ($p_{\text{FC} \times \text{AMF} \times \text{Zeolite}} = 0.0016$; Fig. 2A). A similar pattern was observed for microbial biomass nitrogen (MBN), which reached its maximum value ($50.94 \pm 0.39 \text{ mg kg}^{-1}$) under the best treatment compared with $37.40 \pm 0.42 \text{ mg kg}^{-1}$ in the non-treated ones (Fig. 2B). Both enhanced MBC and MBN reflect an expansion of the active microbial pool and improved substrate availability. The consistent superiority of AMF-inoculated treatments suggests that mycorrhizal colonization stimulated rhizodeposition and microbial interactions, thereby enhancing soil biological turnover under stress conditions.

Soil organic matter (SOM; Fig. 2C) and available soil water (ASW; Fig. 2D) both exhibited clear upward trends with increasing zeolite rate, indicating improved water retention and nutrient buffering capacity with SOM increasing from $10.04 \pm 0.04\%$ to $11.32 \pm 0.04\%$, and ASW elevated from $22.47 \pm 0.23\%$ to $38.90 \pm 0.41\%$ under the combined treatments. Likewise, cation exchange capacity (CEC) peaked at 33.94

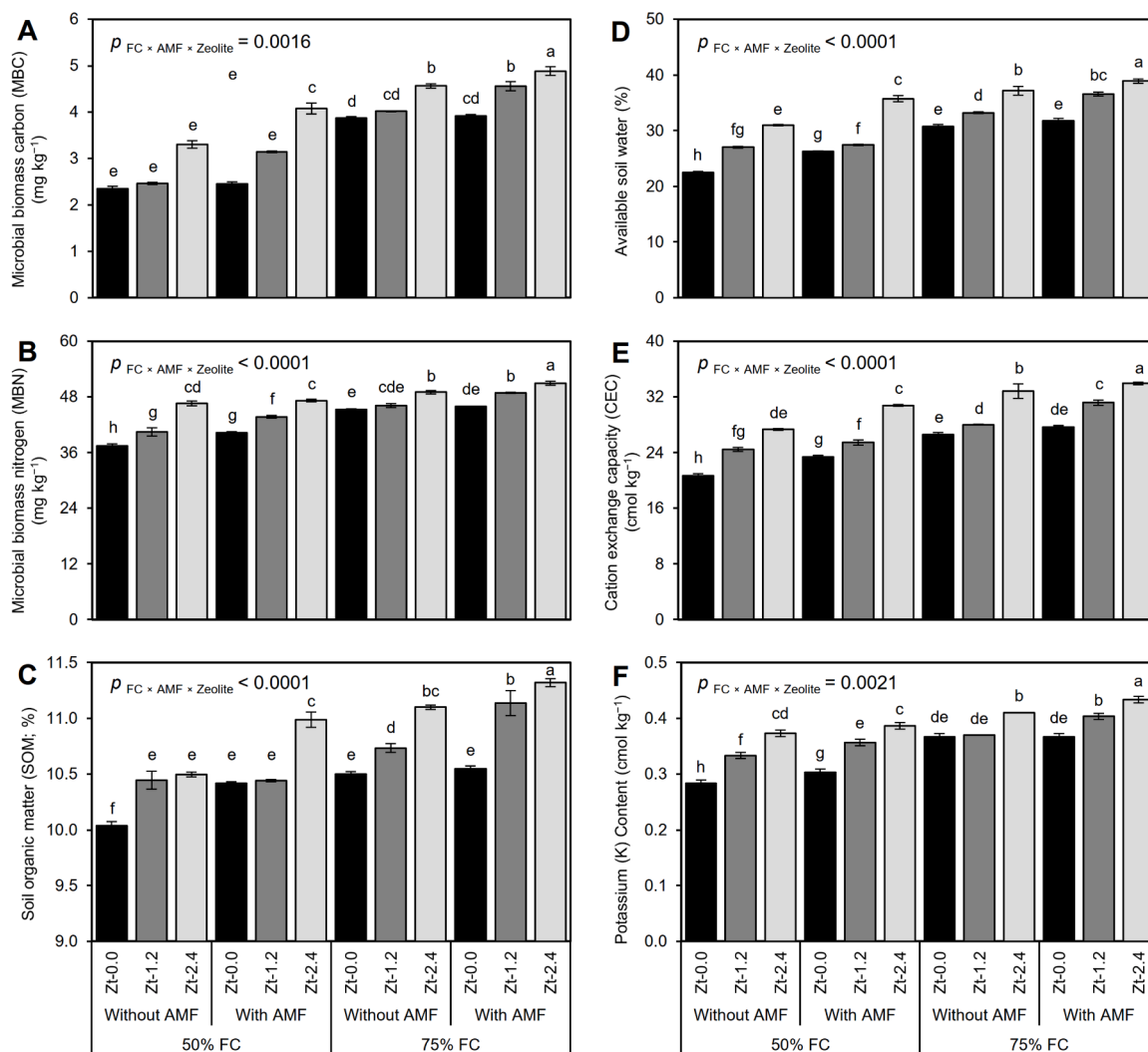


Fig. 2. Effect of field capacity (FC), arbuscular mycorrhizal fungi (AMF), and zeolite on key biochemical and fertility properties of saline soil cultivated with wheat and irrigated with cadmium-contaminated wastewater. (A) Microbial biomass carbon (MBC) (mg kg^{-1}), (B) Microbial biomass nitrogen (MBN) (mg kg^{-1}), (C) Soil organic matter (SOM; %), (D) Available soil water (%), (E) Cation exchange capacity (CEC) (cmol kg^{-1}), and (F) Potassium (K) Content (cmol kg^{-1}). The experiment was arranged in a split-split plot design with two FC levels (50% and 75%) as main plots, two AMF treatments (with and without inoculation) as subplots, and three zeolite application rates (0, 1.2, and 2.4 t ha^{-1}) as sub-subplots under saline soil conditions irrigated with cadmium-contaminated wastewater. Bars present means $\pm$ standard deviation (SD) of three independent biological replicates. Different letters denote statistically significant differences among treatment means according to the three-way interaction ($p_{\text{FC} \times \text{AMF} \times \text{Zeolite}} \leq 0.05$), while the same letters indicate no statistically significant differences.

$\pm 0.17 \text{ cmol kg}^{-1}$, compared to only $20.71 \pm 0.24 \text{ cmol kg}^{-1}$ in the non-amended soil (Fig. 2E). Consequently, zeolite amendment increased the soil's ability to retain exchangeable cations, particularly exchangeable K^+ , which increased from 0.28 ± 0.01 – $0.43 \pm 0.01 \text{ cmol kg}^{-1}$ (Fig. 2F). Collectively, these biochemical responses indicate that zeolite-AMF synergy effectively shifted the soil from a stress-dominated state to a more fertile, biologically active system with enhanced nutrient buffering

capacity under saline-cadmium conditions.

3.4. Effect on soil enzymatic activities

Soil enzymatic activities were highly responsive and sensitive to both biological and chemical experimental amendments, with the strongest stimulation observed under the combined treatment (Table S4). Catalase

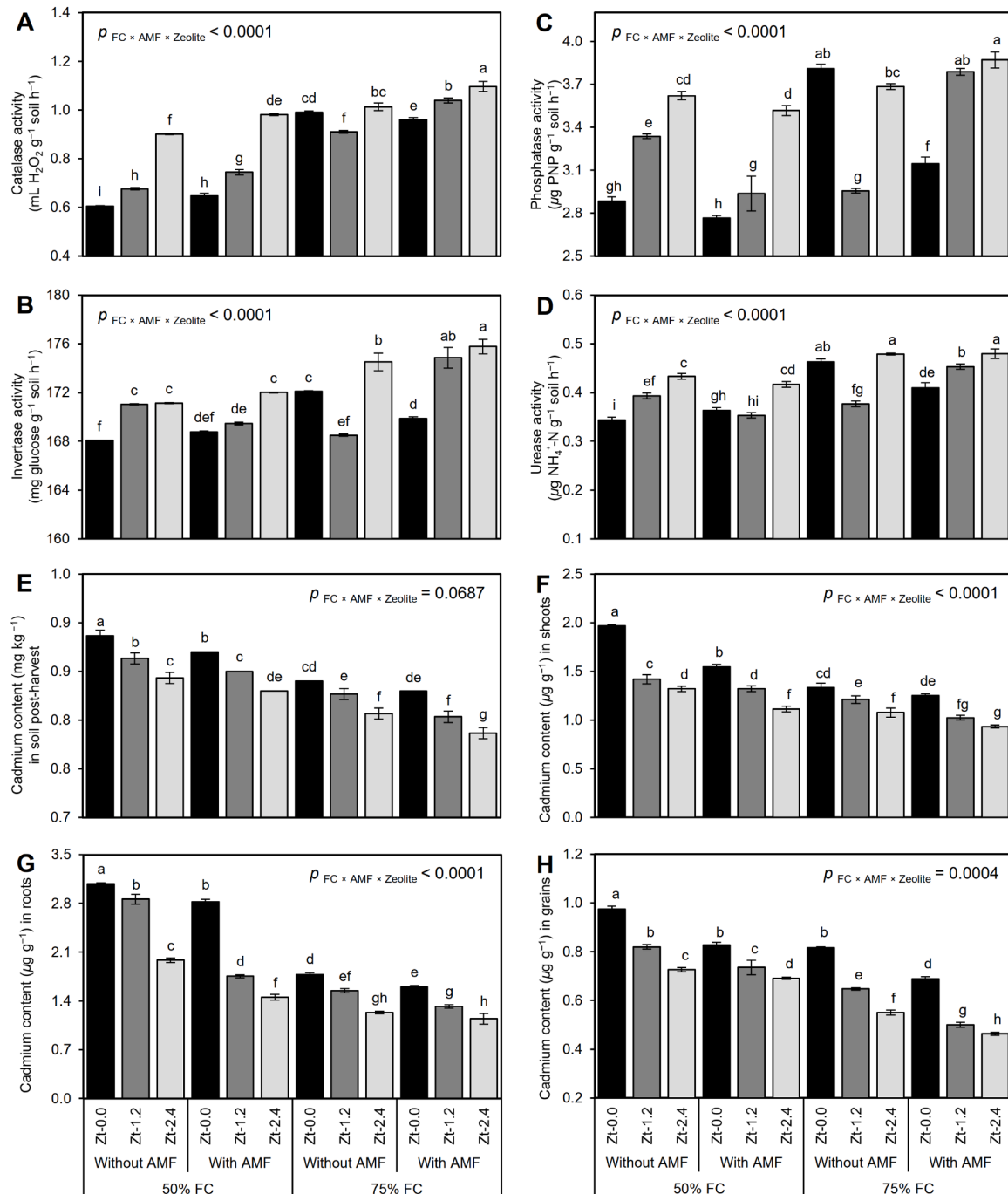


Fig. 3. Effect of field capacity (FC), arbuscular mycorrhizal fungi (AMF), and zeolite on major enzymatic activities and cadmium (Cd) accumulation in saline soil cultivated with wheat and irrigated with cadmium-contaminated wastewater. (A) Catalase activity ($\text{mL H}_2\text{O}_2 \text{ g}^{-1} \text{ soil h}^{-1}$), (B) Invertase activity ($\text{mg glucose g}^{-1} \text{ soil h}^{-1}$), (C) Phosphatase activity ($\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$), (D) Urease activity ($\mu\text{g NH}_4\text{-N g}^{-1} \text{ soil h}^{-1}$), (E) Cd content (mg kg^{-1}) in soil post-harvest, (F) Cd content ($\mu\text{g g}^{-1}$) in roots, (G) Cd content ($\mu\text{g g}^{-1}$) in shoots, and (H) Cd content ($\mu\text{g g}^{-1}$) in grains. The experiment was arranged in a split-split plot design with two FC levels (50% and 75%) as main plots, two AMF treatments (with and without inoculation) as subplots, and three zeolite application rates (0, 1.2, and 2.4 t ha^{-1}) as sub-subplots under saline soil conditions irrigated with cadmium-contaminated wastewater. Bars present means $\pm$ standard deviation (SD) of three independent biological replicates. Different letters denote statistically significant differences among treatment means according to the three-way interaction ($p_{\text{FC} \times \text{AMF} \times \text{Zeolite}} \leq 0.05$), while the same letters indicate no statistically significant differences.

activity increased markedly from $0.61 \pm 0.00 \mu\text{mol H}_2\text{O}_2 \text{ g}^{-1} \text{ soil h}^{-1}$ in the untreated stressed treatment (50% FC, -AMF, 0 zeolite) to 1.10 ± 0.02 under 75% FC + AMF + Zeolite 2.4 t ha^{-1} (Fig. 3A), indicating enhanced oxidative stress buffering capacity. Likewise, invertase activity also peaked at $175.77 \pm 0.61 \text{ mg glucose g}^{-1} \text{ soil h}^{-1}$, compared to 168.08 ± 0.02 in untreated soil (Fig. 3B).

Both phosphatase (Fig. 3C) and urease (Fig. 3D) activities followed similar patterns, with maximum activities of $3.87 \pm 0.06 \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$ and $0.48 \pm 0.01 \mu\text{g NH}_4^+ \text{ g}^{-1} \text{ h}^{-1}$, respectively, under the combined treatments compared with untreated stressed treatment (50% FC, -AMF, 0 zeolite) (2.88 ± 0.03 and 0.34 ± 0.01), reflecting enhanced cycling of phosphorus and nitrogen, respectively. These enzymatic responses confirm that zeolite and AMF jointly enhanced the functional capacity of soil microbial communities, thereby reinforcing nutrient turnover and biochemical resilience under combined salinity and cadmium stress.

3.5. Cadmium residue in saline soil and cultivated wheat

Cadmium (Cd) dynamics were profoundly influenced by zeolite and AMF application (Table S5), with moisture regime exerting an additional modulatory effect, with clear reductions in Cd concentration across soil and plant compartments (Fig. 3E-H). Soil Cd content post-harvest declined progressively from $0.89 \pm 0.01 \text{ mg kg}^{-1}$ in the untreated stressed treatment (50% FC, -AMF, 0 zeolite) to $0.79 \pm 0.01 \text{ mg kg}^{-1}$ under 75% FC + AMF + Zeolite 2.4 t ha^{-1} (Fig. 3E), reflecting the strong sorption capacity of zeolite and its ability to immobilize heavy metals through surface complexation and ion exchange.

More importantly, Cd accumulation in roots, shoots, and grains was substantially suppressed under AMF inoculation and zeolite application, indicating restricted metal uptake and translocation within the plant. Briefly, Cd uptake in roots declined sharply from 3.08 ± 0.01 – $1.14 \pm 0.08 \mu\text{g g}^{-1}$ (Fig. 3F), while shoot Cd decreased from 1.97 ± 0.01 – $0.93 \pm 0.02 \mu\text{g g}^{-1}$ (Fig. 3G). Most importantly, grain Cd was reduced from 0.98 ± 0.01 – $0.46 \pm 0.01 \mu\text{g g}^{-1}$ (Fig. 3H), representing a

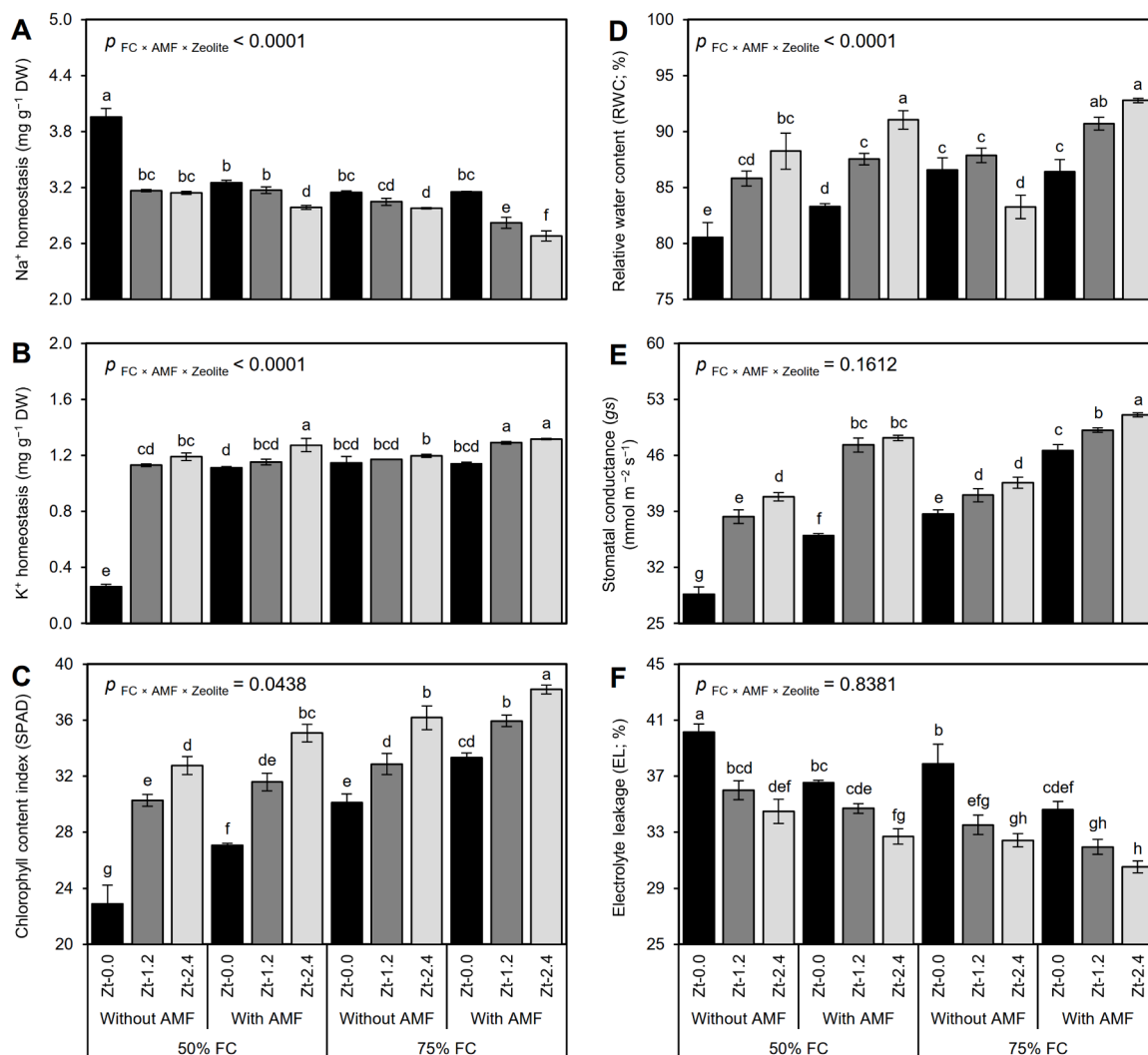


Fig. 4. Effect of field capacity (FC), arbuscular mycorrhizal fungi (AMF), and zeolite on physiological responses and water relations of wheat plants grown in saline soil and irrigated with cadmium-contaminated wastewater. (A) Na^+ homeostasis ($\text{mg g}^{-1} \text{ DW}$), (B) K^+ homeostasis ($\text{mg g}^{-1} \text{ DW}$), (C) Chlorophyll content index (SPAD), (D) Relative water content (RWC; %), (E) Stomatal conductance ($\text{gs; mmol m}^{-2} \text{ s}^{-1}$), and (F) Electrolyte leakage (EL; %). The experiment was arranged in a split-split plot design with two FC levels (50% and 75%) as main plots, two AMF treatments (with and without inoculation) as subplots, and three zeolite application rates (0, 1.2, and 2.4 t ha^{-1}) as sub-subplots under saline soil conditions irrigated with cadmium-contaminated wastewater. Bars present means $\pm$ standard deviation (SD) of three independent biological replicates. Different letters denote statistically significant differences among treatment means according to the three-way interaction ($p_{\text{FC} \times \text{AMF} \times \text{Zeolite}} \leq 0.05$), while the same letters indicate no statistically significant differences.

53% reduction. These findings highlight that zeolite primarily acted as a chemical barrier in the soil, while AMF contributed a biological barrier by regulating metal transport at the root interface.

3.6. Physiological responses and water relations of wheat plants

The physiological performance of wheat grown in saline soil

cultivated with wheat and irrigated with cadmium-contaminated wastewater was improved significantly in response to soil amendments (Table S6), with clear restoration of ionic balance and water status (Fig. 4). Leaf Na^+ concentration declined from $3.95 \pm 0.09 \text{ mg g}^{-1} \text{ DW}$ in stressed plants to $2.68 \pm 0.06 \text{ mg g}^{-1} \text{ DW}$ under zeolite and AMF treatments (Fig. 4A), while K^+ concentration increased from 0.26 ± 0.02 – $1.32 \pm 0.01 \text{ mg g}^{-1} \text{ DW}$ (Fig. 4B), indicating improved

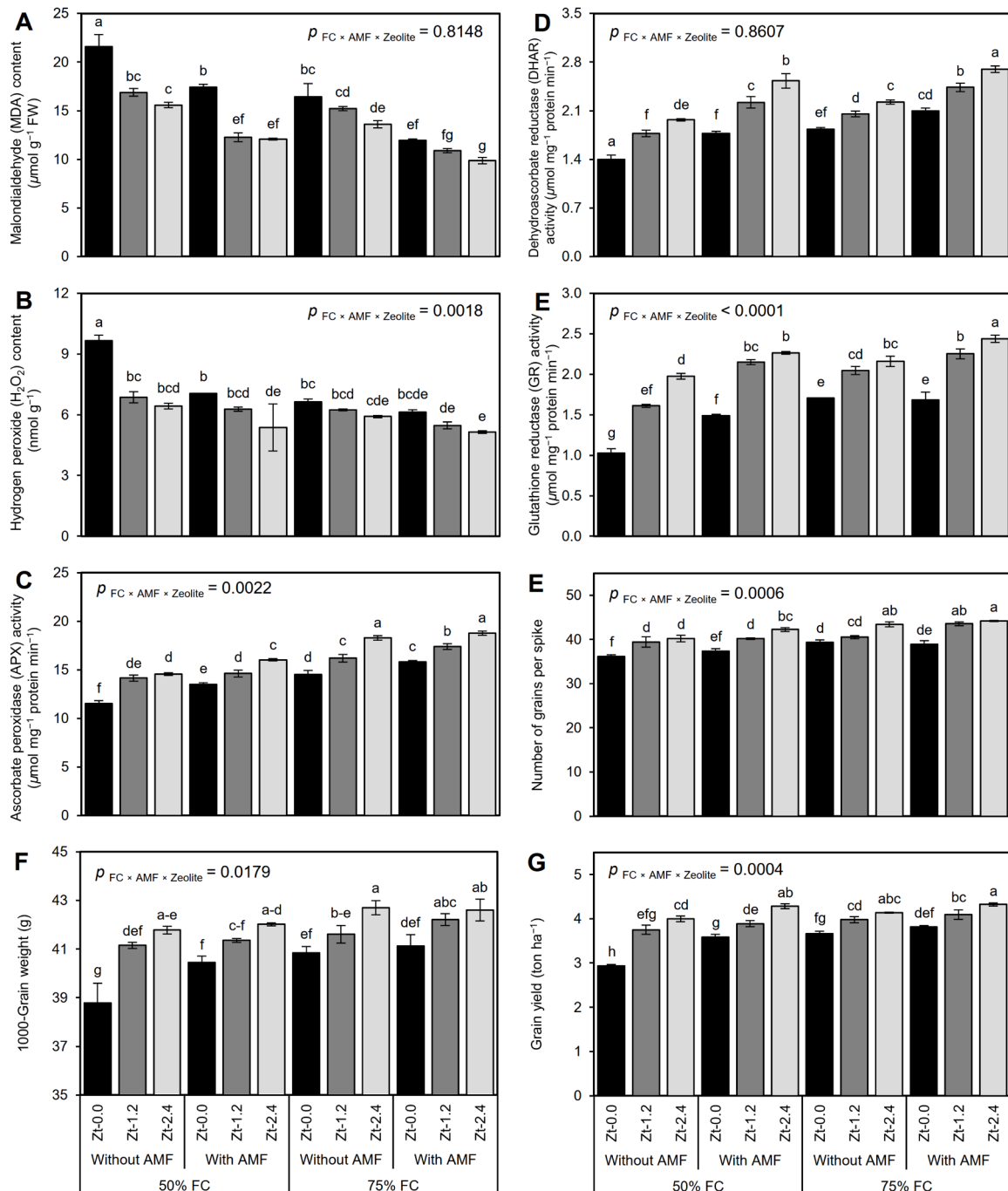


Fig. 5. Effect of field capacity (FC), arbuscular mycorrhizal fungi (AMF), and zeolite on redox homeostasis indicators and yield components of wheat plants grown in saline soil and irrigated with cadmium-contaminated wastewater. (A) Malondialdehyde (MDA) content, (B) Hydrogen peroxide (H_2O_2) content, (C) Ascorbate peroxidase (APX) activity, (D) Dehydroascorbate reductase (DHAR) activity, (E) Glutathione reductase (GR) activity, (F) Number of grains per spike, (G) 1000-Grain weight (g), and (H) Grain yield (ton ha^{-1}). The experiment was arranged in a split-split plot design with two FC levels (50% and 75%) as main plots, two AMF treatments (with and without inoculation) as subplots, and three zeolite application rates (0, 1.2, and 2.4 t ha^{-1}) as sub-subplots under saline soil conditions irrigated with cadmium-contaminated wastewater. Bars present means $\pm$ standard deviation (SD) of three independent biological replicates. Different letters denote statistically significant differences among treatment means according to the three-way interaction ($p_{\text{FC} \times \text{AMF} \times \text{Zeolite}} \leq 0.05$), while the same letters indicate no statistically significant differences.

selectivity of ion uptake and enhanced membrane transport regulation. This shift in Na^+/K^+ balance is a key physiological indicator of salinity tolerance.

Additionally, chlorophyll content, relative water content (RWC), and stomatal conductance (g_s) all increased significantly under the combined treatment. Briefly, chlorophyll content (SPAD) reached 38.18 ± 0.32 under 75% FC + AMF + Zeolite 2.4 t ha^{-1} , compared with only 22.89 ± 1.33 in the untreated stressed treatment (50% FC, -AMF, 0 zeolite) (Fig. 4C). RWC increased from $80.55 \pm 1.30\%$ to $92.77 \pm 0.21\%$ (Fig. 4D), while stomatal conductance elevated from 28.67 ± 0.88 – $51.07 \pm 0.22 \text{ mmol m}^{-2} \text{ s}^{-1}$ (Fig. 4E), reflecting enhanced photosynthetic capacity and improved plant hydration. Parallely, electrolyte leakage declined from $40.15 \pm 0.57\%$ to $30.52 \pm 0.43\%$ (Fig. 4F), indicating greater membrane stability and reduced cellular injury. Together, these data indicate profound recovery of plant water relations and physiological integrity.

3.7. Redox homeostasis indicators of wheat plants

Notably, oxidative stress markers were strongly alleviated by zeolite and AMF, indicating a substantial improvement in plant redox homeostasis (Fig. 5 and Table S7). Lipid peroxidation (as indicated by MDA content) and hydrogen peroxide (H_2O_2) accumulation declined consistently across treatments, suggesting reduced cellular damage and

improved ROS scavenging efficiency. Briefly, MDA content decreased from $21.60 \pm 1.23 \mu\text{mol g}^{-1} \text{ FW}$ in stressed plants to $9.85 \pm 0.33 \text{ nmol g}^{-1}$ under 75% FC + AMF + zeolite 2.4 t ha^{-1} (Fig. 5A), while H_2O_2 declined from 9.67 ± 0.26 – $5.14 \pm 0.07 \mu\text{mol g}^{-1} \text{ FW}$ (Fig. 5B).

On the other hand, antioxidant enzyme activities increased markedly, particularly those associated with the ascorbate-glutathione cycle. Ascorbate peroxidase (APX) activity was increased from 11.52 ± 0.29 – $18.77 \pm 0.22 \mu\text{mol mg}^{-1} \text{ protein min}^{-1}$ (Fig. 5C), dehydroascorbate reductase (DHAR) activity was improved from 1.40 ± 0.07 – $2.70 \pm 0.05 \mu\text{mol mg}^{-1} \text{ protein min}^{-1}$ (Fig. 5D), and glutathione reductase (GR) activity augmented from 1.03 ± 0.05 – $2.44 \pm 0.05 \mu\text{mol mg}^{-1} \text{ protein min}^{-1}$ (Fig. 5E). These results indicate that the integrated soil amendments alleviated external stress while strengthening internal antioxidant defenses through activation of the ascorbate-glutathione cycle, leading to improved redox homeostasis under combined salinity and Cd stress.

3.8. Yield components of wheat plants

Yield components responded positively and consistently to the integrated management strategy (Table S8), confirming that physiological and soil improvements were effectively translated into productivity gains. The number of grains per spike increased from 36.14 ± 0.42 in the untreated stressed treatment (50% FC, -AMF, 0 zeolite) to 44.22

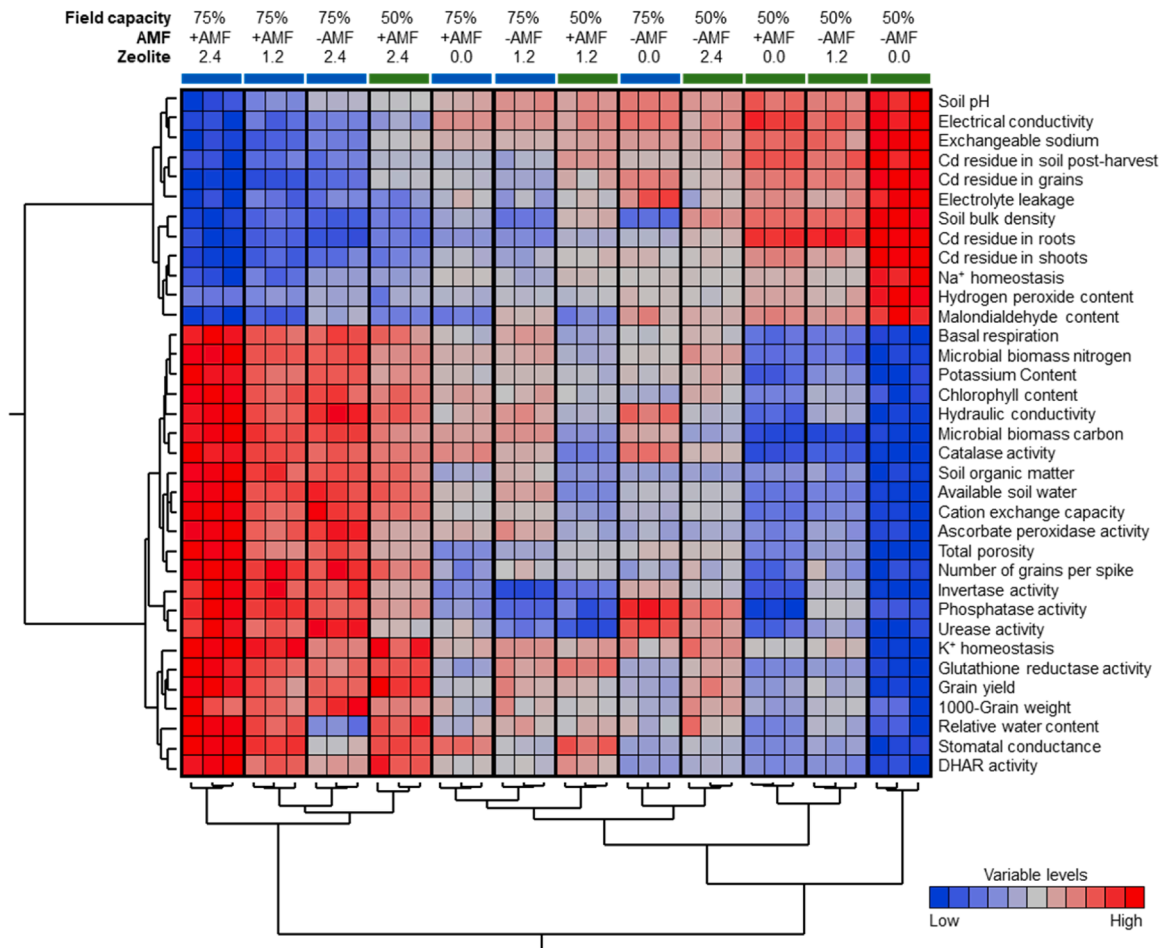


Fig. 6. Hierarchical Cluster Analysis (HCA) and its associated heatmap illustrating the integrated responses of wheat grown under saline soil conditions irrigated with Cd-contaminated wastewater to soil moisture regime, arbuscular mycorrhizal fungi (AMF) inoculation, and zeolite application. Treatments include two field capacity levels (50 and 75% FC), AMF inoculation (with and without), and three zeolite rates (0, 1.2, and 2.4 t ha^{-1}). Colors represent standardized values of the measured growth, physiological, nutritional, and cadmium-related parameters, with blue indicating lower values and red indicating higher values. The heatmap highlights distinct treatment-dependent patterns and synergistic effects of AMF and zeolite, particularly under higher soil moisture, in mitigating salinity and Cd stress and sustaining wheat performance.

± 0.14 under 75% FC + AMF + zeolite 2.4 t ha^{-1} (Fig. 5F). Similarly, 1000-grain weight rose from $38.79 \pm 0.80 \text{ g}$ to $42.60 \pm 0.44 \text{ g}$ under zeolite and AMF treatments (Fig. 5G), reflecting improved assimilate partitioning and grain filling efficiency. Consequently, grain yield showed the strongest response, with the combined treatment consistently outperforming all other combinations. Grain yield reached a maximum of $4.33 \pm 0.04 \text{ t ha}^{-1}$ under 75% FC + AMF + Zeolite 2.4 t ha^{-1} , compared with only $2.93 \pm 0.04 \text{ t ha}^{-1}$ in stressed untreated plots (Fig. 5H), representing a 48% yield increase. This yield enhancement reflects the cumulative benefits of improved soil structure, nutrient availability, water relations, ionic balance, and oxidative stress regulation.

3.9. HCA reflects the effects of zeolite and mycorrhiza on wheat stress responses

The heatmap presented in Fig. 6 provides an integrative overview of how soil moisture regime, AMF inoculation, and zeolite application interact to regulate wheat performance and stress-related attributes under saline, Cd-contaminated wastewater irrigation. Hierarchical cluster analysis (HCA) and its associated heatmap reveal a clear separation between treatments, indicating strong treatment-driven shifts across the measured variables, rather than random variability, confirming the robustness of the experimental factors.

Notably, the most favorable responses cluster under conditions combining higher field capacity (75% FC), AMF inoculation, and moderate-to-high zeolite rates (1.2 and 2.4 t ha^{-1}) (Fig. 6). These treatments are consistently associated with improvements in plant growth, nutrient status, and physiological performance, alongside reductions in Cd accumulation and stress indicators. In contrast, non-inoculated treatments at lower soil moisture (50% FC), particularly without zeolite, highlight the compounded negative effects of salinity, Cd toxicity, and water limitation.

HCA analysis (Fig. 6) suggests a strong synergistic interaction between zeolite and AMF. Zeolite-amended treatments show a progressive shift as application rate increases, indicating dose-dependent mitigation of salinity and Cd stress. This pattern is especially pronounced when zeolite is combined with AMF, supporting the notion that zeolite improves the soil chemical environment through cation exchange, Cd immobilization, and salinity buffering, thereby enhancing AMF colonization efficiency and functionality. Improved AMF performance, in turn, likely promotes better root exploration, nutrient uptake, and ionic homeostasis, which is reflected in the coordinated improvement of multiple plant traits captured in the heatmap.

Notably, the contrast between 50% and 75% FC treatments underscores the importance of adequate soil moisture in stress mitigation strategies. At 75% FC, even moderate zeolite inputs appear sufficient to shift several parameters toward favorable ranges, whereas under 50% FC, positive responses are weaker and more fragmented (Fig. 6). This indicates that water availability modulates the effectiveness of both biological (AMF) and mineral (zeolite) amendments, likely by influencing mass flow of nutrients, microbial activity, and Cd mobility in the rhizosphere.

4. Discussion

The current study demonstrates that the combined application of arbuscular mycorrhizal fungi (AMF) and zeolite under optimized soil moisture conditions represents an effective and multifunctional strategy for remediating saline soils irrigated with Cd-contaminated wastewater, with clear consequences for soil health, crop productivity, and food safety. The observed improvements in soil physicochemical properties, including reductions in pH, electrical conductivity, and exchangeable sodium, suggest that zeolite may act as a powerful ion-regulating agent capable of buffering excess salts and improving soil chemical

equilibrium (Ferretti et al., 2018). These actions are consistent with the high CEC and porous crystalline structure of zeolite, which enables selective adsorption of Na^+ and heavy metals while releasing beneficial cations such as Ca^{2+} and K^+ (Eroglu et al., 2017; Ferretti et al., 2018). Notably, zeolite efficiency was enhanced at higher soil moisture (75%), suggesting that adequate hydration enhanced diffusion, ion-exchange kinetics, and salt displacement from the rhizosphere.

Beyond chemical remediation, the strong stimulation of microbial biomass, basal respiration, and soil enzymatic activities suggests an essential biological restoration of soil functioning. AMF inoculation possibly played a key role in this process by enhancing root-microbe interactions, stimulating rhizodeposition, and promoting microbial niche formation under abiotic stress conditions. AMF are well known to improve soil aggregation, carbon turnover, and nutrient cycling, particularly in degraded or saline soils (Chandrasekaran et al., 2021; Wahab et al., 2023). The concurrent increase in catalase, invertase, phosphatase, and urease activities suggests that the soil system shifted from a stress-dominated, metabolically constrained state to a more active and functionally resilient microbial ecosystem (Qin et al., 2020). Such biological recovery is critical for long-term soil sustainability, as enzymatic processes directly regulate carbon sequestration and nitrogen and phosphorus availability (Nannipieri et al., 2012).

Additionally, our findings showed that soil physical structure quality was significantly improved by zeolite and AMF, particularly under the higher moisture regime. The improvements in soil structure, including reduced bulk density, increased porosity, and enhanced saturated hydraulic conductivity, further demonstrate that zeolite and AMF not only modified soil chemistry but also reshaped the physical architecture of the soil. Zeolite particles likely acted as aggregation supporting agents with a potential antimicrobial role (Król et al., 2022), while AMF hyphae contributed to aggregate stabilization through physical entanglement and glomalin secretion (Ji et al., 2024). These structural and physical changes of saline soil are agronomically important because enhanced pore connectivity enhances leaching of excess salts, facilitates root penetration, and improves gas exchange (Bronick and Lal, 2005). From an environmental perspective, the observed structural recovery suggests that zeolite and AMF did not just alter soil chemistry but fundamentally reshaped soil physical architecture. Such physical recovery increases soil resilience against secondary salinization and reduces the risk of soil degradation under long-term Cd-contaminated wastewater irrigation.

A particularly important outcome of this study was the substantial reduction in Cd accumulation in wheat tissues, especially in edible grains. Cd concentrations in roots, shoots, and grains were markedly reduced, by up to approximately 53%, following AMF inoculation and zeolite application, indicating restricted Cd uptake and translocation within the plant (Gao et al., 2023; Liu et al., 2026). Grain Cd concentration decreased from 0.98 to 0.46 mg kg^{-1} under the combined treatment, highlighting the effectiveness of the integrated remediation strategy in limiting Cd transfer into edible plant tissues. Zeolite likely contributed to this effect through Cd immobilization via surface complexation and ion-exchange mechanisms, consistent with previous reports on aluminosilicate minerals in heavy metal remediation (Khater and Ghareib, 2021; Viana et al., 2025). Simultaneously, AMF may have acted as a biological barrier by regulating metal uptake at the root interface through Cd sequestration within fungal structures and modification of root transporter activity (Zhao et al., 2024).

Despite this substantial reduction, the final grain Cd concentration (0.46 mg kg^{-1}) remained above the maximum permissible limit established by several international food safety standards, including the Codex Alimentarius guideline value of 0.2 mg kg^{-1} for wheat grain. Therefore, although the integrated zeolite-AMF treatment significantly mitigated Cd accumulation and reduced the potential risk of Cd entry into the human food chain, the obtained grain Cd levels may still pose food safety concerns under highly contaminated irrigation conditions. These findings suggest that additional remediation strategies, improved

wastewater management, or longer-term soil rehabilitation practices may be required to further reduce grain Cd concentrations to fully acceptable safety thresholds. Nevertheless, the observed reduction remains agronomically and environmentally important, considering that Cd is a non-essential and highly toxic element associated with kidney dysfunction, skeletal damage, carcinogenic risks, and reproductive disorders in humans (Genchi et al., 2020; Kumar and Sharma, 2019). Overall, the integrated zeolite-AMF approach represents a promising and eco-friendly strategy for substantially limiting Cd translocation from polluted soils into crop tissues under saline and Cd-stressed conditions.

At the plant level, the physiological performance of wheat grown in saline soil cultivated with wheat and irrigated with cadmium-contaminated wastewater was improved significantly in response to soil amendments. The restoration of Na^+/K^+ homeostasis, chlorophyll content, relative water content, and stomatal conductance indicates that soil remediation translated directly into improved physiological performance. Salinity and Cd stresses are known to disrupt membrane integrity, inhibit photosynthesis, and impair water relations (Gupta and Huang, 2014). The reduction in electrolyte leakage and oxidative stress markers observed in this study reflects improved membrane stability and cellular integrity, which are essential for maintaining redox homeostasis and metabolic activity under stress. Lipid peroxidation and hydrogen peroxide accumulation declined consistently across combined AMF and zeolite treatments, particularly under sufficient irrigation regimes, suggesting reduced cellular damage and improved ROS scavenging efficiency. Furthermore, the strong activation of the ascorbate-glutathione cycle demonstrates that integrated soil amendments enhanced internal antioxidant defense systems, enabling plants to efficiently detoxify reactive oxygen species and maintain redox homeostasis (Abdelaal et al., 2024; Mohammadi et al., 2025). Similar findings have been reported recently in wheat and other crops exposed to salinity or heavy metals under AMF or zeolite amendment regimes (Abdelaal et al., 2024; Mohammadi et al., 2025).

Eventually, these corresponding soil and plant-level enhancements were reflected in substantial gains in yield and yield components. The observed increases in grain number, grain weight, and final yield indicate that the integrated management strategy not only alleviated stress symptoms but also restored productive capacity under highly unfavorable conditions. In other words, this yield enhancement reflects the cumulative benefits of improved soil structure, nutrient availability, water relations, ionic balance, and oxidative stress regulation. Zeolite and AMF play some synergistic roles in enhancing crop yield and productivity, particularly under abiotic stress conditions such as salinity, drought, and nutrient limitation (Beslemes et al., 2023; Mahmoud et al., 2023). Zeolite contributes primarily through improving soil physico-chemical properties, including cation exchange capacity, water retention, and nutrient availability, which collectively promote root growth and nutrient uptake efficiency, ultimately leading to higher biomass accumulation and yield (Aslani et al., 2021; Mondal et al., 2021). Several field studies have demonstrated that zeolite application significantly enhances cereal productivity by mitigating salinity stress and improving soil structural and nutritional status, particularly when integrated with organic amendments or improved agronomic practices (Mahmoud et al., 2023).

In parallel, AMF enhance crop productivity through biological mechanisms, including improved phosphorus and nitrogen acquisition, stimulation of photosynthetic activity, and modulation of plant metabolic pathways associated with stress tolerance (Beslemes et al., 2023). Previous studies confirmed that AMF inoculation significantly increases crop yields across diverse agroecosystems by promoting biomass production and resource-use efficiency, particularly under abiotic stress conditions (Wu et al., 2022). Collectively, these findings indicate that integrating zeolite with AMF under optimized irrigation represents a promising and sustainable strategy for mitigating both salinity and Cd stress, while also delivering agronomic benefits in terms of plant physiological performance and crop productivity simultaneously. Although

zeolite and AMF substantially reduced Cd bioavailability and plant uptake, continued long-term irrigation with Cd-contaminated wastewater may still contribute to gradual Cd accumulation in soil systems over time. Therefore, periodic monitoring of soil Cd status remains essential under sustained wastewater irrigation practices.

From a sustainability perspective, the findings of this study are particularly significant because saline and metal-contaminated soils are expanding globally due to climate change, poor irrigation practices, and industrial pollution (Ijaz et al., 2024; Yu et al., 2025). We proved that combining natural minerals such as zeolite with biological inoculants like AMF represents a low-cost, environmentally safe, and scalable solution for reclaiming degraded soils while safeguarding food quality. Collectively, the zeolite-AMF-moisture synergy created a multifunctional remediation system that simultaneously improved soil chemical balance, biological activity, physical structure, plant physiological resilience, and heavy metal safety. This integrative approach aligns well with the principles of climate-smart and sustainable agriculture, offering a practical pathway to maintain crop productivity in agroecosystems increasingly threatened by salinity and metal contamination.

5. Conclusion

The current study provides strong evidence that combining zeolite and AMF is a multifunctional, sustainable approach to improving wheat productivity and safety in saline soils irrigated with Cd-contaminated wastewater. The proposed integrated treatment restored soil chemical balance by diminishing salinity and exchangeable sodium, enhanced biological functioning by stimulating microbial biomass and enzymatic activity, and improved soil physical structure by reducing bulk density and increasing porosity and saturated hydraulic conductivity. These improvements collectively provide a more favorable soil environment that supports enhanced nutrient availability, water retention, and photosynthesis functioning. Notably, zeolite and AMF significantly reduced Cd residue in plant tissues, particularly in grains, thereby minimizing the risk of heavy metal entry into the human food chain. At the plant level, the remediation strategy improved ionic homeostasis, water relations, and redox homeostasis, ultimately translating into substantial yield gains. From an agronomic and environmental perspective, zeolite-AMF integration offers a practical, eco-friendly, and scalable solution for reclaiming degraded saline soils and mitigating heavy metal risks in agroecosystems increasingly challenged by water quality deterioration and climate-driven soil degradation.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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CRediT authorship contribution statement

Asmaa M. Badr: Software. **Hagagy A. Ahmed:** Validation. **Khadiga Alharbi:** Funding acquisition. **Youzhi Feng:** Investigation. **Alaa El-Dein Omara:** Visualization. **Yasser Nehela:** Writing – original draft, Visualization. **Tarek Alshaal:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Emad M. Hafez:** Conceptualization. **Wenlong Xu:** Data curation.

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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2026.120395](https://doi.org/10.1016/j.ecoenv.2026.120395).

Data availability

Data will be made available on request.

References

1986. A.KluteMethods of soil analysis. Part 1. Physical and mineralogical methodsAmerican Society of Agronomy, Inc.Madison, Wisconsin.
- Abdallah, M.M., Suo, C., Cui, Y., Ullah, R.H., Nhung, H.H., Li, L., Liu, C., 2026. Arbuscular mycorrhizal fungi as integrative modulators of plant tolerance to drought, salinity, and heavy metal stress: mechanistic insights and future directions. *J. Genet. Eng. Biotechnol.* 24, 100636. <https://doi.org/10.1016/J.JGEB.2025.100636>.
- Abdelal, K., Alaskar, A., Hafez, Y., 2024. Effect of arbuscular mycorrhizal fungi on physiological, bio-chemical and yield characters of wheat plants (*Triticum aestivum* L.) under drought stress conditions. *BMC Plant. Biol.* 24, 1119. <https://doi.org/10.1186/S12870-024-05824-9/FIGURES/7>.
- Aiad, M.A., Amer, M.M., Khalifa, T.H.H., Shabana, M.M.A., Zoghdan, M.G., Shaker, E.M., Eid, M.S.M., Ammar, K.A., Al-Dhumri, S.A., Kheir, A.M.S., 2011. Combined application of compost, zeolite and a raised bed planting method alleviate salinity stress and improve cereal crop productivity in arid regions. *Agronomy* 11. <https://doi.org/10.3390/AGRONOMY1111>.
- Al-Kofahi, S.D., Almrhleh, R.Y., Sawalhah, M.N., Othman, Y.A., 2025. Yield and physiological responses of wheat (*Triticum aestivum* L.) in relation to different levels of nutrient. *South. Afr. J. Bot.* 184, 1051–1061. <https://doi.org/10.1016/J.SAJB.2025.07.020>.
- Anas, M., Khattak, W.A., Fahad, S., Alrawiq, N., Alrawiq, H.S., Abdelsalam, N.R., Jaremo, M., Quraishi, U.M., 2025. Mitigating heavy metal pollution in agriculture: A multi-omics and nanotechnology approach to safeguard global wheat production. *J. Hazard. Mater. Adv.* 17, 100584. <https://doi.org/10.1016/J.HAZADV.2024.100584>.
- Anderson, J.P.E., 1982. In: Page, A.L. (Ed.), *Soil Respiration. Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*, pp. 831–871. <https://doi.org/10.2134/AGRONMONOGR9.2.2ED.C41>.
- Aryal, J.P., Becerra Lopez-Lavalle, L.A., El-Naggar, A.H., 2025. Crop Loss Due to Soil Salinity and Agricultural Adaptations to It in the Middle East and North Africa Region. *Resour* 2025 14, 14. <https://doi.org/10.3390/RESOURCES14090139>.
- Asgari, K., Cornelis, W.M., 2015. Heavy metal accumulation in soils and grains, and health risks associated with use of treated municipal wastewater in subsurface drip irrigation. *Environ. Monit. Assess.* 187, 410. <https://doi.org/10.1007/S10661-015-4565-8/FIGURES/3>.
- Aslani, P., Davari, M., Mahmoodi, M.A., Hosseinpanahi, F., Khaleghpanah, N., 2021. Effect of zeolite and nitrogen on some basic soil properties and wheat yield in potato-wheat rotation. *Agric. Eng.* 44, 97–119. <https://doi.org/10.22055/AGEN.2021.37397.1603>.
- Bajji, M., Kinet, J.M., Lutts, S., 2002. The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant. Growth Regul.* 36, 61–70. <https://doi.org/10.1023/A:1014732714549>.
- Barnes, J., 2022. *Staple Security: Bread and Wheat in Egypt*, 1st ed, Staple Security. Duke University Press. <https://doi.org/10.1215/9781478023111>.
- Barrs, H., Weatherley, P., 1962. A Re-Examination of the Relative Turgidity Technique for Estimating Water Deficits in Leaves. *Aust. J. Biol. Sci.* 15, 413–428. <https://doi.org/10.1071/bi9620413>.
- Ben Hassen, T., El Bilali, H., Allahyari, M.S., Baya Chatti, C., 2025. Traditional irrigation knowledge for sustainable water resource management in arid Environments: Insights from the MENA region. *J. Arid. Environ.* 231, 105466. <https://doi.org/10.1016/J.JARIDENV.2025.105466>.
- Beslemes, D., Tigka, E., Roussis, I., Kakabouki, I., Mavroeidis, A., Vlachostergios, D., 2023. Effect of Arbuscular Mycorrhizal Fungi on Nitrogen and Phosphorus Uptake Efficiency and Crop Productivity of Two-Rowed Barley under Different Crop Production Systems. *Plants* 2023 12, 12. <https://doi.org/10.3390/PLANTS12091908>.
- Blake, G.R., Hartge, K.H., 2018. Bulk density. In: Klute, A. (Ed.), *Methods of Soil Analysis, Part 1: Physical and Mineralogical Methods*. John Wiley & Sons, Ltd, pp. 363–375. <https://doi.org/10.2136/sssabookser5.1.2ed.c13>.
- Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. *Geoderma* 124, 3–22. <https://doi.org/10.1016/J.GEODERMA.2004.03.005>.
- Burns, R.G., 1982. Enzyme activity in soil: Location and a possible role in microbial ecology. *Soil. Biol. Biochem.* 14, 423–427. [https://doi.org/10.1016/0038-0717\(82\)90099-2](https://doi.org/10.1016/0038-0717(82)90099-2).
- Chandrasekaran, M., Boopathi, T., Manivannan, P., 2021. Comprehensive Assessment of Ameliorative Effects of AMF in Alleviating Abiotic Stress in Tomato Plants. *J. Fungi* 2021 7, 7. <https://doi.org/10.3390/JOF7040303>.
- Cui, S., Zhou, Q., Chao, L., 2007. Potential hyperaccumulation of Pb, Zn, Cu and Cd in enduring plants distributed in an old smelter, northeast China. *Environ. Geol.* 51, 1043–1048. <https://doi.org/10.1007/S00254-006-0373-3/FIGURES/2>.
- Dangi, P., Chaudhary, N., Paul, A., Sharma, A., Dutta, L., Razdan, R., 2023. Pigmented Wheat: Nutrition Scenario and Health Benefits. In: Bangar, S.P., Maqsood, S., Siroha, A.K. (Eds.), *Food Chemistry, Function and Analysis: Pigmented Cereals and Millets*. The Royal Society of Chemistry, pp. 1–26. <https://doi.org/10.1039/9781837670291-00001>.
- Dick, R.P., 2011. *Methods of Soil Enzymology*. SSSA Book Series. Wiley. <https://doi.org/10.2136/SSSABOOKSER9>.
- Dorigan de Matos Furlanetto, A.L., Kaziuk, F.D., Martinez, G.R., Donatti, L., Merlin Rocha, M.E., dos Santos, A.L.W., Floh, E.I.S., Cadena, S.M.S.C., 2021. Mitochondrial bioenergetics and enzymatic antioxidant defense differ in Paraná pine cell lines with contrasting embryogenic potential. *Free. Radic. Res.* 55, 255–266. <https://doi.org/10.1080/10715762.2021.1921172>.
- Du, Z., Bramlage, W.J., 1992. Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. *J. Agric. Food Chem.* 40, 1566–1570. <https://doi.org/10.1021/JF00021A018>.
- El-maghraby, F.M., Shaker, E.M., Elbagory, M., Omara, A.E.D., Khalifa, T.H., 2024. The Synergistic Impact of Arbuscular Mycorrhizal Fungi and Compost Tea to Enhance Bacterial Community and Improve Crop Productivity under Saline-Sodic Condition. *Plants* 2024 13, 13. <https://doi.org/10.3390/PLANTS13050629>.
- Eroglu, N., Emekci, M., Athanassiou, C.G., 2017. Applications of natural zeolites on agriculture and food production. *J. Sci. Food Agric.* 97, 3487–3499. <https://doi.org/10.1002/JFSA.8312>.
- Feng, J., Zhang, H., Gao, Z., Gao, S., Cui, J., Wang, H., Pan, H., Yang, Q., Yang, Z., Sun, Y., Lou, Y., Zhuge, Y., 2025. A novel modified zeolite enhances Cadmium immobilisation in weakly alkaline soil: Synergistic mechanisms of thermal activation and humic acid functionalization. *Ecotoxicol. Environ. Saf.* 305, 119229. <https://doi.org/10.1016/J.ECOENV.2025.119229>.
- Ferretti, G., Di Giuseppe, D., Faccini, B., Coltorti, M., 2018. Mitigation of sodium risk in a sandy agricultural soil by the use of natural zeolites. *Environ. Monit. Assess.* 190, 646. <https://doi.org/10.1007/S10661-018-7027-2/FIGURES/5>.
- Gao, Y., An, T., Kuang, Q., Wu, Y., Liu, S., Liang, L., Yu, M., Macrae, A., Chen, Y., 2023. The role of arbuscular mycorrhizal fungi in the alleviation of cadmium stress in cereals: A multilevel meta-analysis. *Sci. Total. Environ.* 902, 166091. <https://doi.org/10.1016/J.SCITOTENV.2023.166091>.
- Genchi, G., Sinicropi, M.S., Lauria, G., Carocci, A., Catalano, A., 2020. The Effects of Cadmium Toxicity. *Int. J. Environ. Res. Public. Heal.* 2020 17, 17. <https://doi.org/10.3390/IJERPH17113782>.
- Guan, S.Y., Zhang, D., Zhang, Z., 1986. *Soil enzyme and its research methods*. Agricultural 274–297.
- Guo, J., Chen, Y., Zhao, Y., Tian, H., Jiang, Y., Zhang, Y., 2025. Molecular mechanisms of wheat acclimation to soil degradation: Insights into salt stress, heavy metal contamination, and nutrient deficiency. *Environ. Exp. Bot.* 237, 106193. <https://doi.org/10.1016/J.JENVEXPBOT.2025.106193>.
- Gupta, B., Huang, B., 2014. Mechanism of Salinity Tolerance in Plants: Physiological, Biochemical, and Molecular Characterization. *Int. J. Genom.* 2014, 701596. <https://doi.org/10.1155/2014/701596>.
- Hesse, P.R., 1971. publisher) Ltd. *A Textbook of Soil Chemical Analysis*. Juan murry, London.
- Ijaz, S., Iqbal, J., Abbasi, B.A., Tufail, A., Ullah, Z., Yaseen, T., Ali, I., Uddin, S., Iqbal, R., 2024. Heavy metal-polluted arable land and its consequences: a global scenario, in: Biochar-Assisted. Remediation of Contaminated Soils Under Changing Climate. Elsevier, pp. 71–99. <https://doi.org/10.1016/b978-0-443-21562-9.00003-7>.
- Jackson, M.L., 1958. *Soil Chemical Analysis*. Englewood Cliffs, NJPrentice-hall, INC.
- Jackson, M.L., 1967. *Soil Chemical Analysis*. Prentice-Hall of India Pvt. Ltd. Scientific Research Publishing, New Delhi.
- Javaid, A., Munir, N., Abideen, Z., Siddiqui, Z.S., Yong, J.W.H., 2024. The role of natural and synthetic zeolites as soil amendments for mitigating the negative impacts of abiotic stresses to improve agricultural resilience. *Plant. Stress.* 14, 100627. <https://doi.org/10.1016/J.STRESS.2024.100627>.
- Jebara, S., Jebara, M., Limam, F., Aouani, M.E., 2005. Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress. *J. Plant. Physiol.* 162, 929–936. <https://doi.org/10.1016/J.JPLPH.2004.10.005>.
- Ji, L., Chen, X., Huang, C., Tan, W., 2024. Arbuscular mycorrhizal hyphal networks and glomalin-related soil protein jointly promote soil aggregation and alter aggregate hierarchy in Calcaric Regosol. *Geoderma* 452, 117096. <https://doi.org/10.1016/J.GEODERMA.2024.117096>.
- Khan, Y., Shah, S., Li, D., Yang, F., 2025. Arbuscular mycorrhizal fungi-mediated abiotic stress tolerance: Emerging roles in nutrient exchange, antioxidant defence, and

- hormonal crosstalk. *Plant. Stress.* 18, 101068. <https://doi.org/10.1016/J.STRESS.2025.101068>.
- Khater, H.M., Ghareib, M., 2021. Utilization of alkaline Aluminosilicate activation in heavy metals immobilization and producing dense hybrid composites. *Arab. J. Sci. Eng.* 46, 6333–6348. <https://doi.org/10.1007/S13369-020-05065-6/FIGURES/17>.
- Klute, A., Page, A.L., 1982. Methods of soil analysis. Part 2 - Chemical and Microbiological Analysis, Second Edition. American Society of Agronomy.
- Król, M., Sygula-Cholewińska, J., Sawoszczuk, T., 2022. Zeolite-Supported Aggregate as Potential Antimicrobial Agents in Gypsum Composites. *Materials* 15, 3305. <https://doi.org/10.3390/MA15093305>.
- Kumar, A., Kumar, Reetesh, Singh, P., Kalaichelvan, S., Santos-Villalobos, S. de los, Kumar, N., Kumar, Fernando, L., Rajeev, Solanki, M.K., Joshi, N.C., Babalola, O.O., 2025. Emerging Role of Arbuscular Mycorrhizal Fungi in Sustainable Agriculture: From Biology to Field Application. *Microbiologyopen* 14, e70082. <https://doi.org/10.1002/MB03.70082>.
- Kumar, S., Sharma, A., 2019. Cadmium toxicity: effects on human reproduction and fertility. *Rev. Environ. Health* 34, 327–338. <https://doi.org/10.1515/REVEH-2019-0016/MACHINEREADABLECITATION/RIS>.
- Lin, T.H., Rao, M.Y., Lu, H.W., Chiou, C.W., Lin, S.T., Chao, H.W., Zheng, Z.L., Cheng, H. C., Lee, T.M., 2018. A role for glutathione reductase and glutathione in the tolerance of *Chlamydomonas reinhardtii* to photo-oxidative stress. *Physiol. Plant.* 162, 35–48. <https://doi.org/10.1111/PPL.12622>.
- Ling, Q., Huang, W., Jarvis, P., 2011. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana*. *Photosynth. Res.* 107, 209–214. <https://doi.org/10.1007/S11120-010-9606-0/FIGURES/4>.
- Liu, X., Mak, M., Babla, M., Wang, F., Chen, G., Veljanoski, F., Wang, G., Shabala, S., Zhou, M., Chen, Z.H., 2014. Linking stomatal traits and expression of slow anion channel genes HvSLAH1 and HvSLAC1 with grain yield for increasing salinity tolerance in barley. *Front. Plant. Sci.* 5, 117452. <https://doi.org/10.3389/FPLS.2014.00634/TEXT>.
- Liu, J., Qiu, G., Chen, X., Zhang, Junbo, Fu, C., Fu, Q., Chen, A., Zhang, Jun, Tong, W., Guo, B., 2026. Restricting roles of arbuscular mycorrhizal fungi in cadmium uptake and translocation in tomato. *J. Hazard. Mater.* 501, 140866. <https://doi.org/10.1016/J.JHAZMAT.2025.140866>.
- Mahmoud, A.W.M., Rashad, H.M., Esmail, S.E.A., Alsamadany, H., Abdeldaym, E.A., 2023. Application of Silicon, Zinc, and Zeolite Nanoparticles—A Tool to Enhance Drought Stress Tolerance in Coriander Plants for Better Growth Performance and Productivity. *Plants* 2023 12, 12. <https://doi.org/10.3390/PLANTS12152838>.
- Mohammadi, H., Rostamzadeh, S., Ghorbanpour, M., 2025. Exploring the influence of zeolite and nano-zeolite on growth, physiological and phytochemical parameters of *Datura stramonium* L. in cadmium-contaminated soil. *3 Biotech* 15, 266. <https://doi.org/10.1007/S13205-025-04442-Y/FIGURES/2>.
- Mondal, M., Biswas, B., Garai, S., Sarkar, S., Banerjee, H., Brahmachari, K., Bandyopadhyay, P.K., Maitra, S., Brestic, M., Skalicky, M., Ondrisik, P., Hossain, A., 2021. Zeolites Enhance Soil Health, Crop Productivity and Environmental Safety. *Agronomy* 11, 11. <https://doi.org/10.3390/agronomy11030448>.
- Mushtaq, G., Agrawal, S., Kushwah, A., Kumar, A., Lone, R., 2025. Cadmium toxicity in plants and its remediation management: A review. *Plant Stress* 16, 100894. <https://doi.org/10.1016/J.STRESS.2025.100894>.
- Mussarat, M., Jamal, W.A., Muhammad, D., Ahmad, M., Saleem, A., Khan, S., Aman, F., Bibi, H., Shah, W.A., Dawar, K., Ul Akbar, N., Mian, I.A., Waheed, M., Ali, I., Zia, A., Malik, W., 2021. Risk of heavy metals accumulation in soil and wheat grains with waste water irrigation under different NPK levels in alkaline calcareous soil. *PLoS. One* 16, 0258724. <https://doi.org/10.1371/JOURNAL.PONE.0258724>.
- Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornasier, F., Moscatelli, M.C., Marinari, S., 2012. Soil enzymology: Classical and molecular approaches. *Biol. Fertil. Soils* 48, 743–762. <https://doi.org/10.1007/S00374-012-0723-0/FIGURES/3>.
- Navarro-Torre, S., Garcia-Caparrós, P., Nogales, A., Abreu, M.M., Santos, E., Cortinhas, A.L., Caperta, A.D., 2023. Sustainable agricultural management of saline soils in arid and semi-arid Mediterranean regions through halophytes, microbial and soil-based technologies. *Environ. Exp. Bot.* 212, 105397. <https://doi.org/10.1016/J.ENVEXPBOT.2023.105397>.
- Paz, A.M., Amezketa, E., Canfora, L., Castanheira, N., Falsone, G., Gonçalves, M.C., Gould, I., Hristov, B., Mastorilli, M., Ramos, T., Thompson, R., Costantini, E.A.C., 2023. Salt-affected soils: field-scale strategies for prevention, mitigation, and adaptation to salt accumulation. *Ital. J. Agron.* 18, 2166. <https://doi.org/10.4081/IJA.2023.2166>.
- Qin, M., Zhang, Q., Pan, J., Jiang, S., Liu, Y., Bahadur, A., Peng, Z., Yang, Y., Feng, H., 2020. Effect of arbuscular mycorrhizal fungi on soil enzyme activity is coupled with increased plant biomass. *Eur. J. Soil. Sci.* 71, 84–92. <https://doi.org/10.1111/EJSS.12815>.
- Rashid, A., Schutte, B.J., Ulery, A., Deyholos, M.K., Sanogo, S., Lehnhoff, E.A., Beck, L., 2023. Heavy Metal Contamination in Agricultural Soil: Environmental Pollutants Affecting Crop Health. *Agron* 2023 13, 13. <https://doi.org/10.3390/AGRONOMY13061521>.
- Richards, L.A., 1954. Diagnosis and Improvement of Saline and Alkali Soils (Agriculture Handbook No. 60). UNITED STATES DEPARTMENT OF AGRICULTURE, Washington D. C.
- Schneider, K., Turrión, M.B., Gallardo, J.F., 2000. Modified method for measuring acid phosphatase activities in forest soils with high organic matter content. *Commun. Soil. Sci. Plant. Anal.* 31, 3077–3088. <https://doi.org/10.1080/00103620009370651>.
- Seilsepour, M., Rashidi, M., Khabbazi, B.G., 2009. Prediction of soil exchangeable sodium percentage based on soil sodium adsorption ratio. *Am. J. Agric. Environ. Sci.* 5, 1–4.
- Setia, R., Verma, S.L., Marschner, P., 2012. Measuring microbial biomass carbon by direct extraction - Comparison with chloroform fumigation-extraction. *Eur. J. Soil. Biol.* 53, 103–106. <https://doi.org/10.1016/J.EJSOBI.2012.09.005>.
- Shah, G.M., Farooq, U., Shabbir, Z., Guo, J., Dong, R., Bakhat, H.F., Wakeel, M., Siddique, A., Shahid, N., 2023. Impact of Cadmium Contamination on Fertilizer Value and Associated Health Risks in Different Soil Types Following Anaerobic Digestate Application. *Toxics* 2023 11, 11. <https://doi.org/10.3390/TOXICS11121008>.
- Silva, B.M., da Silva, E.A., de Oliveira, G.C., Ferreira, M.M., Serafim, M.E., 2014. Plant-available soil water capacity: estimation methods and implications. *Rev. Bras. Ciência do Solo* 38, 464–475. <https://doi.org/10.1590/S0100-06832014000200011>.
- Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., 2020. Methods of soil analysis. Part 3, Chemical methods. John Wiley & Sons.
- Sumner, M.E., Miller, W.P., 2018. Cation exchange capacity and exchange coefficients. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (Eds.), *Methods of Soil Analysis, Part 3: Chemical Methods*. John Wiley & Sons, Ltd, pp. 1201–1229. <https://doi.org/10.2136/sssabookser5.3.c40>.
- Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant. Sci.* 151, 59–66. [https://doi.org/10.1016/S0168-9452\(99\)00197-1](https://doi.org/10.1016/S0168-9452(99)00197-1).
- Viana, R. da S.R., Chagas, J.K.M., Paz-Ferreiro, J., Figueiredo, C.C. de, 2025. Enhanced remediation of heavy metal-contaminated soils using biochar and zeolite combinations with additives: A meta-analysis. *Environ. Pollut.* 367, 125617. <https://doi.org/10.1016/J.ENVPOL.2024.125617>.
- Wahab, A., Muhammad, M., Munir, A., Abdi, G., Zaman, W., Ayaz, A., Khizar, C., Reddy, S.P.P., 2023. Role of Arbuscular Mycorrhizal Fungi in Regulating Growth, Enhancing Productivity, and Potentially Influencing Ecosystems under Abiotic and Biotic Stresses. *Plants* 2023 12, 12. <https://doi.org/10.3390/PLANTS12173102>.
- Wang, H., Krug, L.J., Li, Z., Sun, P., Qian, X., Dittert, K., 2025. Zeolite-induced enhancement of soil cation exchange capacity reduces nitrogen gaseous emissions and improves nitrate retention under urea fertilization. *Environ. Technol. & Innov.* 40, 104567. <https://doi.org/10.1016/J.ETI.2025.104567>.
- Wolf, B., 1982. A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. *Commun. Soil. Sci. Plant. Anal.* 13, 1035–1059. <https://doi.org/10.1080/00103628209367332>.
- Wu, S., Shi, Z., Chen, X., Gao, J., Wang, X., 2022. Arbuscular mycorrhizal fungi increase crop yields by improving biomass under rainfed condition: A meta-analysis. *PeerJ* 10, e12861. <https://doi.org/10.7717/PEERJ.12861/SUPP-5>.
- Yu, J., Chen, Z., Gao, W., He, S., Xiao, D., Fan, W., Huo, M., Nugroho, W.A., 2025. Global trends and prospects in research on heavy metal pollution at contaminated sites. *J. Environ. Manag.* 383, 125402. <https://doi.org/10.1016/J.JENVMAN.2025.125402>.
- Zhang, M., Jia, R., Ma, M., Yang, T., Sun, Q., Li, M., 2023. Versatile wheat gluten: functional properties and application in the food-related industry. *Crit. Rev. Food Sci. Nutr.* 63, 10444–10460. <https://doi.org/10.1080/10408398.2022.2078785>.
- Zhao, S., Yan, L., Kamran, M., Liu, S., Riaz, M., 2024. Arbuscular Mycorrhizal Fungi-Assisted Phytoremediation: A Promising Strategy for Cadmium-Contaminated Soils. *Plants* 13, 3289. <https://doi.org/10.3390/PLANTS13233289>.
- Zheng, X., Lin, H., Du, D., Li, G., Alam, O., Cheng, Z., Liu, X., Jiang, S., Li, J., 2024. Remediation of heavy metals polluted soil environment: A critical review on biological approaches. *Ecotoxicol. Environ. Saf.* 284, 116883. <https://doi.org/10.1016/J.ECOENV.2024.116883>.
- Zhou, H., Shi, H., Yang, Y., Feng, X., Chen, X., Xiao, F., Lin, H., Guo, Y., 2024. Insights into plant salt stress signaling and tolerance. *J. Genet. Genom.* 51, 16–34. <https://doi.org/10.1016/J.JGG.2023.08.007>.