



Enhancing rice productivity in wastewater-irrigated saline Cd-contaminated soils using microbial-nanoparticle synergy

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ABSTRACT

Soil salinity and cadmium (Cd) contamination pose significant threats to agricultural productivity and food security, particularly in rice-growing regions. This study investigates the synergistic effects of plant growth-promoting rhizobacteria (PGPRs) (*Pseudomonas koreensis*, *Bacillus coagulans*, and *Pseudomonas stutzeri*) and selenium nanoparticles (SeNPs) in remediating saline Cd-contaminated soils and enhancing rice (*Oryza sativa* L.) performance. Over two consecutive growing seasons (2022–2023), the combined application of PGPRs and SeNPs significantly improved soil health, reducing soil pH from 8.50 to 8.02 and electrical conductivity (EC_e) from 5.97 to 4.01 dS m⁻¹, while increasing soil organic matter (SOM) by 6.5 % and cation exchange capacity (CEC) by 25.6 %. The treatment also reduced soil Cd content by 34.6 %, from 0.81 to 0.53 mg kg⁻¹, and decreased Cd accumulation in rice roots, shoots, and seeds by 56.7 %, 65.0 %, and 50.0 %, respectively, ensuring safer rice grain production. Furthermore, SeNPs significantly enhanced selenium (Se) content in rice shoots and seeds, with Se levels increasing from 0.55 to 1.47 µg g⁻¹ in shoots and from 0.01 to 0.51 µg g⁻¹ in seeds, highlighting their role in improving rice nutritional quality. Physiological analyses revealed enhanced photosynthetic pigment concentrations, with chlorophyll a increasing by 112.3 % and carotenoids by 213.6 %, alongside a 101.9 % increase in superoxide dismutase (SOD) activity under the combined treatment. These improvements translated into a 25.0 % increase in grain yield, from 5.76 to 7.24 ton ha⁻¹, and a 21.4 % increase in 1000-grain weight. The findings highlight the efficacy of PGPRs and SeNPs in mitigating oxidative stress, improving nutrient uptake, reducing Cd toxicity, and enhancing rice productivity under combined salinity and Cd stress. This study provides a novel, eco-friendly

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strategy for sustainable soil remediation and crop production in contaminated environments, offering significant implications for global food security and agricultural sustainability.

1. Introduction

The variable climatic conditions, accelerated industrial and agricultural activities, and population explosion exerted severe pressure on current water resources, causing water scarcity and compromising sustainable agriculture (Mishra et al., 2012). Irrigating arable lands with untreated wastewater is a common practice worldwide in several countries to overcome this issue (Wang et al., 2024). The poor farmers rely on sewage because of its availability around the clock, sufficient nutrients, and organic matter, which reduces synthetic fertilizers use (Natasha et al., 2023).

Continuous wastewater irrigation leads to the accumulation of heavy metals in agricultural soils, particularly cadmium (Cd), which deteriorates soil quality and crop productivity (Yan et al., 2020). The irrigation water used in this study contained $0.09 \text{ mg Cd L}^{-1}$, exceeding the FAO's recommended maximum threshold of 0.01 mg L^{-1} for irrigation water (Ayers and Westcot, 1985) by nine-fold, thereby qualifying as Cd-contaminated. Cd concentrations in wastewater-irrigated soils often exceed regulatory limits; for instance, studies report Cd levels ranging from $0.5\text{--}5 \text{ mg kg}^{-1}$ in contaminated agricultural soils, far above the WHO permissible limit of 0.1 mg kg^{-1} for agricultural land (WHO, 2006) and the EPA threshold of 0.4 mg kg^{-1} for residential soils (EPA, 2023). In rice-growing regions, Cd accumulation in grains can reach $0.2\text{--}1.5 \text{ mg kg}^{-1}$, surpassing the Codex Alimentarius standard of 0.4 mg kg^{-1} for rice (Codex, 2019). Chronic Cd exposure via contaminated food poses severe health risks, including renal dysfunction, osteoporosis, and cancer (Satarug, 2018; Reyes-Hinojosa et al., 2019). These thresholds underscore the urgency of remediating Cd-contaminated soils to ensure food safety and environmental sustainability.

Moreover, wastewater also contains sufficient soluble salts, which cause soil salinization after accumulating on the soil surface after evaporation and shallow groundwater salinity through leaching (Wang et al., 2024). The higher accumulation of salts in the soil also resulted in poor soil structure, which reduced microbial communities, plant growth, biomass, photosynthesis, and nutrient uptake, leading to yield loss (Alharbi et al., 2022a). Soil salinization increased Cd bioavailability through competition between salt cations and Cd for sorption sites, allowing Cd dissolution from soil particles, which enhanced Cd accumulation in edible crops, posing severe threats to humans (Wang et al., 2024).

The inoculation of plant growth-promoting rhizobacteria (PGPRs) is a gentle and innovative sustainable remediation strategy for improving crop production under salinity and Cd stress. These beneficial microbes, such as *Pseudomonas* (*P. stutzeri*, *P. koreensis*) and *Bacillus* (*B. coagulans*, *B. mycoides*) and others species, have been tested to improve plant growth and soil quality under soil salinity (Nehela et al., 2021; Omara et al., 2022; Alharbi et al., 2022b) and Cd stress (Ma et al., 2023; Alshaal et al., 2024), causing negative impacts on plant physiological traits (Thongnok et al., 2022; Alharbi et al., 2022a). Moreover, these bacterial strains also produce numerous phytohormones, improved nitrogen fixation, secretion of exopolysaccharide substances (EPS), and improved water retention, favoring plant growth by ameliorating salt (Alharbi et al., 2022a) and Cd stress (Shi et al., 2024).

The foliar applications of mineral nanoparticles have been tested under abiotic stress and promote plant growth. Among various mineral nutrients, selenium (Se) is a crucial fertilizer in enhancing plant growth and crop yield (Feng et al., 2021). For instance, selenium nanoparticles (SeNPs) improved photosynthesis formation, gas exchange attributes, synthesis of secondary metabolites, osmoprotectants accumulation, reduced free radical formation and reduced oxidative stress (Bano et al., 2021). It also protects plants against heavy metals induced oxidative stress by synthesizing selenoenzymes, improving the antioxidant activities, protecting cell walls, and scavenging free radicals (Zhu et al., 2022).

The combination of PGPRs and SeNPs creates a synergistic system that enhances plant resilience through complementary mechanisms. PGPRs improve soil health and plant growth by producing phytohormones (e.g., IAA), solubilizing nutrients, and reducing metal bioavailability through biosorption and sequestration (Ma et al., 2023). Meanwhile, SeNPs mitigate oxidative stress by up-regulating antioxidant enzymes (SOD, CAT) and forming inert metal-Se complexes that reduce Cd uptake (Zhu et al., 2022). When combined, PGPRs enhance the bioavailability of SeNPs by improving root absorption and colonization, while SeNPs protect PGPRs from metal toxicity, enabling sustained microbial activity (Alshaal et al., 2024). This dual action amplifies soil remediation and plant growth under stress, as demonstrated by Shang et al. (2024), who reported a 50 % greater reduction in Cd accumulation with combined PGPR-SeNP treatment compared to individual applications.

Globally, rice (*Oryza sativa* L.) is a staple crop critical for food security, but its productivity is severely threatened by soil salinity, which can reduce yields by 20–70 % depending on stress intensity and growth stage (Munns and Tester, 2008; Shereen et al., 2014). Salinity impairs germination, with even moderate levels ($EC_e = 4\text{--}6 \text{ dS m}^{-1}$) decreasing seedling establishment by 30–40 % (Zeng and Shannon, 2000), while chronic exposure disrupts photosynthesis, induces oxidative stress, and degrades soil structure, exacerbating long-term yield losses (Rengasamy, 2010; Hasanuzzaman et al., 2018). These challenges are compounded in wastewater-irrigated soils, where cadmium (Cd) contamination further suppresses growth and grain quality (Hafez et al., 2019; Alharbi et al., 2022a). Thus, developing cost-effective strategies to mitigate combined salinity and Cd stress is imperative for sustainable rice production (Alharbi et al., 2022b; Wang et al., 2024).

Previous studies highlighted the potential of PGPRs and SeNPs as a novel approach to enhancing crop productivity and soil fertility from salt (Bano et al., 2021) and Cd-affected soils (Thongnok et al., 2022). We postulated that the conjunction of *P. stutzeri*, *P. koreensis*, and *B. coagulans* altered the physico-chemical properties of soil and colonized rice plants and reduced salt and Cd uptake. In contrast, foliar SeNP applications improved rice growth and yield and prevented rice plants from salt and Cd toxicity. Additionally, foliar SeNPs

provision improved Se content in rice grain and quality attributes compared to their sole applications.

Hence, this study aims to evaluate the synergistic effects of PGPRs and SeNPs in alleviating saline Cd-contaminated soil and enhancing rice productivity. The research focuses on addressing the dual challenges of soil salinity and heavy metal contamination, which severely impair soil health and crop yields. By combining microbial consortia (*Pseudomonas koreensis*, *Bacillus coagulans*, and *Pseudomonas stutzeri*) with SeNPs, the study seeks to improve soil physicochemical properties, reduce Cd bioavailability, and enhance rice physiological and biochemical traits under stress conditions. The justification for this approach lies in the urgent need for sustainable and cost-effective strategies to remediate contaminated soils and ensure food security, particularly in regions where rice is a staple crop. The findings are expected to provide a novel, eco-friendly solution for improving crop resilience and yield in saline and Cd-polluted environments, contributing to global efforts in sustainable agriculture.

2. Materials and methods

2.1. Bacterial strains and growth conditions

A lab trial was performed to study the capability of PGPRs to augment the seed germination of rice (*Oryza sativa* L., cv. Sakha 105) under different salinity and cadmium concentrations. For this objective, the trial was executed in a completely randomized design. Six PGPR strains, i.e., *Pseudomonas koreensis* MG209738, *Azotobacter chroococcum* SARS 10, *Azospirillum lipoferum* SP2, *Bacillus coagulans* NCAIM B 1086, *Enterobacter cloacae* KX034162, *Pseudomonas stutzeri* were obtained from the Department of Agricultural Microbiology, Soils, Water, and Environment Research Institute (SWERI), ARC, Egypt. For better growth, these bacterial strains were inoculated on the King's B broth medium (glycerol 20 g L⁻¹, tryptone 20 g L⁻¹, MgSO₄ 1.5 g L⁻¹, K₂HPO₄ 1.5 g L⁻¹, and pH 7.4) on a rotary shaker at 150 rpm and 30 °C for three days.

2.2. Evaluation of PGPR strains for NaCl and Cd tolerance

Six bacterial strains were tested in five concentrations of NaCl solution with electrical conductivity (EC) of 0, 2, 4, 6, and 8 dS m⁻¹ to assess indole acetic acid (IAA) production and seed germination (%). Each trial was replicated three times. Also, these six bacterial strains were tested in five concentrations of CdCl₂•2.5 H₂O (0, 5, 10, 15 and 20 mg Cd L⁻¹) and the growth of bacteria was calculated by optical density (OD) at 540 nm by a UV-160A spectrophotometer (Shimadzu, Kyoto, Japan). The IAA production (µg mL⁻¹) was determined using broth medium supplemented with 1 g L⁻¹ filter-sterilized L-tryptophan as IAA precursor in the existence of salinity and Cd stress and incubated at 30 °C on a shaker at 200 rpm for three days (Hafez et al., 2019). In addition, final germination

Table 1

Traits of experimental soil and irrigation water (wastewater) during the two growing seasons (2022 and 2023) of rice plants.

Soil	2022	2023
pH (1:2.5 soil:water suspension)	8.53 ± 0.01	8.51 ± 0.02
EC _e of soil paste extract [†] (dS m ⁻¹)	6.22 ± 0.11	6.03 ± 0.13
Soil organic matter (%)	1.02 ± 0.03	1.07 ± 0.02
Exchangeable sodium percentage (%)	19.36 ± 0.21	19.08 ± 0.19
Texture grade	Clayey	Clayey
Ca ²⁺ (meq L ⁻¹)	7.02 ± 0.09	9.08 ± 0.08
Mg ²⁺ (meq L ⁻¹)	5.32 ± 0.08	6.02 ± 0.06
Na ⁺ (meq L ⁻¹)	26.98 ± 0.11	27.23 ± 0.15
K ⁺ (meq L ⁻¹)	0.34 ± 0.01	0.35 ± 0.01
HCO ₃ ⁻ (meq L ⁻¹)	4.36 ± 0.06	3.48 ± 0.05
Cl ⁻ (meq L ⁻¹)	25.98 ± 0.65	25.49 ± 0.53
SO ₄ ²⁻ (meq L ⁻¹)	14.89 ± 0.12	14.47 ± 0.14
Available-N (mg kg ⁻¹)	9.25 ± 0.08	10.11 ± 0.08
Available-P (mg kg ⁻¹)	8.51 ± 0.09	9.02 ± 0.08
Available-K (mg kg ⁻¹)	357 ± 11	368 ± 14
	2022	Water
pH	7.07 ± 0.01	7.21 ± 0.01
EC (dS m ⁻¹)	0.54 ± 0.01	0.56 ± 0.01
SAR	1.33 ± 0.02	1.38 ± 0.02
Na ⁺ (meq L ⁻¹)	1.99 ± 0.03	2.05 ± 0.03
Cl ⁻ (meq L ⁻¹)	3.89 ± 0.05	3.95 ± 0.06
NH ₄ ⁺ (meq L ⁻¹)	1.69 ± 0.03	1.72 ± 0.02
SO ₄ ²⁻ (meq L ⁻¹)	0.11 ± 0.02	0.13 ± 0.01
Cd (mg L ⁻¹) [‡]	0.09 ± 0.002	0.09 ± 0.001
Pb (mg L ⁻¹)	0.08 ± 0.001	0.08 ± 0.001
Ni (mg L ⁻¹)	0.16 ± 0.01	0.17 ± 0.02

[†] Electrical conductivity.

[‡]The recommended maximum level of Cd in irrigation water set by FAO is 0.01 mg L⁻¹.

percentage (FGP, %) was determined using 10 well-rinsed rice seeds by 70 % (v/v) ethanol then dripped into bacterial suspensions nightlong pre-germinated in sterile 15-cm Petri plates. Five concentrations of NaCl and Cd stress were distributed in plates and therefore incubated at 20 °C in a dark place. Control treatment was dripped into distilled water. All Petri dishes were tightly closed to avoid ventilation out through the 10-day trial time. Day-to-day germination count was performed. FGP (%) was measured based on [Alsaeedi et al. \(2018\)](#), by the equation:

$$FGP = \frac{TNG}{TNP} \times 100$$

where TNG is the total number of germinated seeds, and TNP is the total number of planted seeds.

2.3. Field experiment

An open field trial was conducted in saline-affected soil in Gharbia Governorate, Egypt (latitude: 30° 58.24' N; longitude: 31° 10.01' E). The study aimed to evaluate the effects of superior microbial consortia (*Pseudomonas koreensis* MG209738, *Bacillus coagulans* NCAIM B 1086, and *Pseudomonas stutzeri*) and selenium nanoparticles (SeNPs) on the properties of salt-affected soil irrigated with Cd-contaminated wastewater, as well as on the growth and productivity of rice (*Oryza sativa* L., cv. Sakha 105) during the 2022 and 2023 summer seasons. Rice seedlings were transplanted on June 6 and harvested on December 27. Agricultural practices for rice cultivation followed the guidelines of the Rice Research and Training Center, ARC. Rice seedlings were sprayed with SeNPs at a concentration of 25 mg L⁻¹ three times (based on previous studies, i.e., ([Feng et al., 2021](#); [Bano et al., 2021](#); [Zhu et al., 2022](#)), at 3, 5, and 7 weeks after transplanting, using a volume of 400 L ha⁻¹, while untreated plants (CK treatment) were sprayed with tap water. The experimental design was a split-plot arranged in randomized complete blocks with three replicates. The superior microbial consortia (*Pseudomonas koreensis* MG209738, *Bacillus coagulans* NCAIM B 1086, and *Pseudomonas stutzeri*) were selected based on their performance in IAA production and their ability to enhance rice seed germination under salinity and Cd stress. The selected PGPRs were incubated for two days at 30°C at a 1:1:1 ratio. The inoculum was prepared by mixing 900 mL of 10⁸ CFU mL⁻¹ with 900 g of sterilized peat carrier. Seeds of rice were inoculated with the prepared inoculum at a rate of 2 kg ha⁻¹. The SeNPs were obtained from the Soils, Water, and Environment Research Institute (SWERI), Sakha, Kafr El-Sheikh, Egypt. Rice seeds were soaked in fresh water for one day and incubated for another day at a rate of 120 kg ha⁻¹. Recommended fertilization rates, pesticides, and herbicides were applied according to the Egyptian Ministry of Agriculture. Irrigation was carried out using Cd-contaminated wastewater sourced from the Kitchener drain ([Table 1](#)). Three representative soil samples were collected from each experimental plot at a depth of 0–30 cm before planting using an auger. The samples were air-dried and sieved through a 2-mm mesh for physical and chemical analyses ([Table 1](#)).

2.4. Soil physicochemical assay

[Sumner and Miller \(2018\)](#) three representative soil samples were gathered from each experimental plot at depth of 0–30 cm after harvesting by an auger to assess physicochemical properties. Soil samples were prepared in a soil-water suspension (1:2.5) to assess soil reaction by a pH meter (Genway 3510, Cambridgeshire, UK). However, electrical conductivity (EC_e; dS m⁻¹), cation exchange capacity (CEC; cmolc kg⁻¹), Na⁺ and K⁺ ions (meq L⁻¹) were assessed in a soil paste extract by an EC meter (Jenway 4310, Genway, Cambridgeshire, UK) ([Sparks et al., 1996](#)), ammonium acetate (pH 7.0) procedure ([Sumner and Miller, 2018](#)), and Atomic Absorption Spectrophotometer (AAS, PerkinElmer 3300, Shelton, USA) ([Sparks, 1996](#)), respectively. The exchangeable sodium percentage (ESP) in soil depending on sodium adsorption ratio (SAR) was computed as described by [Richards \(1954\)](#) using the following formula:

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

In addition, other soil samples were air-dried and passed through 20- and 100-mesh sieves to assess soil organic matter (SOM) using potassium dichromate oxidation spectrophotometric method ([Sparks et al., 2020](#)), soil bulk density (g cm⁻³) was assessed by the cylinder procedure ([Sparks, 1996](#)), total porosity (%) was assessed by ([Sparks et al., 1996](#)), and hydraulic conductivity (cm day⁻¹) was assessed by ([Sparks, 1996](#)). Microbial biomass carbon (MBC; mg kg⁻¹ soil) and nitrogen (MBN; mg kg⁻¹ soil) in soil were assessed by the chloroform fumigation-extraction procedure ([Brookes et al., 1985](#)) and ([Wu et al., 1990](#)), it was fumigated 20.0 g of dry soil with ethanol-free chloroform for a day. Fumigated and non-fumigated soils were mixed with 80 mL of 0.5 M K₂SO₄ by shuddering for 30 min on a reciprocating mover at 40 sequences for 60 second and filtered (soil: water = 1:4). The TOC analyzer was utilized to assess the C and N in the extracts. Soil basal respiration (mg CO₂ 100 g⁻¹ dry soil 24 h⁻¹) was assessed by NaOH-trapping glucose after a 10-day incubation period using procedure of ([Kheyrodin et al., 2012](#)). After 60 days of sowing, bacteriological activity (Log cfu g⁻¹ soil) was assessed by the total count of bacteria, *Azotobacter*, and *Bacillus* sp. in soil samples, by the King's B agar medium and modified Ashby's media, following the methods ([Abd-el-Malek et al., 1979](#)).

2.5. Soil enzymes assay

At 60 days after sowing, air-dried soil samples passed through 20 and 100 mesh sieves were used for determining the activity of catalase (EC 1.11.1.6; mg KMnO₄ g⁻¹ day⁻¹), invertase (EC 3.2.1.26; mg glucose g⁻¹ day⁻¹), acid phosphatase (EC 3.1.3.2; mg phenol kg⁻¹ day⁻¹), and urease activity (EC 3.5.1.5; mg NH₄⁺-N g⁻¹ day⁻¹) by the method of [Burns and Dick \(2002\)](#), [Dick \(2011\)](#), and [Schneider et al. \(2000\)](#), respectively. The absorbance of supernatants was assessed at 405, 508, 578, and 240 nm by the UV-160A

spectrophotometer (Shimadzu, Kyoto, Japan), respectively.

2.6. Assessment of Cd uptake and accumulation in rice plants

At harvest, contents of extractable soil Cd (mg kg^{-1}) and plant Cd (root and shoot; $\mu\text{g g}^{-1}$) were determined by Atomic Absorption Spectrophotometer (AAS, PerkinElmer 3300, Shelton, USA) after DTPA-extraction of soil Cd and digestion of plant tissues using HNO_3 and H_2O_2 mixture in a 5:3 ratio (Sparks, 1996). The bioconcentration factor (BCF), translocation factor (TF), and bioaccumulation factor (BAC) were measured by the following equations:

$$\text{BCF} = \text{Cd in roots} / \text{Cd in test soil (Yoon et al., 2006)}$$

$$\text{TF} = \text{Cd in shoots} / \text{Cd in roots (Cui et al., 2007)}$$

$$\text{BAC} = \text{Cd in leaves} / \text{Cd in test soil (Li et al., 2007)}$$

2.7. Quantification of selenium content in plant tissue

The total selenium (Se) content in the shoot and seeds was quantified following the method outlined by Kovács et al. (2023). In brief, 20 mg of powdered sample was placed into a 150-mL Kjeldahl tube and digested using a 3:2 mixture of HNO_3 and H_2O_2 . To convert selenate to selenite, an additional digestion step was performed by adding 20 mL of 6 M HCl to the cooled sample, which was then heated to 100 °C for 60 minutes. The digested samples were diluted to a final volume of 50 mL using Milli-Q water. Total Se content was analyzed using a hydride generation atomic fluorescence spectrometer (PSA 10.055 Millennium Excalibur System, PS Analytical Ltd., United Kingdom). Sodium borohydride (1.4 % m/v) served as the reducing agent to produce volatile hydrogen selenide (H_2Se). To ensure accuracy and traceability of Se measurements the IAEA-336 (Lichen CRM) was used as a Certified Reference Material.

2.8. Quantification of Na and K ions (mg g^{-1}) in aerial parts

At 60 days after sowing, the oven-dried leaf samples were selected and crushed in mortar. This crushed leaf tissues (20 mg) was then applied to a combination of HNO_3 and HClO_4 . The material was then dried at 70 °C for 4 h and then at 100 °C for 3 h. This material digest was then utilized to assess the uptake of Na and K by the Atomic Absorption Spectrophotometer (AAS, PerkinElmer 3300, Shelton, USA) according to (Sparks, 1996).

2.9. Leaf area

At 60 days after sowing, ten leaf samples were obtained from each experimental plot from at the panicle initiation stage, then rinsed with distilled water, and dehydrated. The leaf area was assessed by a leaf area meter (Model LI-COR, Inc., Lincoln, NE, USA).

2.10. Photosynthetic pigments

At 60 days after sowing, chlorophyll a, chlorophyll b, and carotenoids as mg g^{-1} of fresh weight were assessed by procedure of Lichtenthaler (1987) according to the absorbance at 663, 645, and 470 nm, respectively, by the UV-160A spectrophotometer (Shimadzu, Kyoto, Japan).

$$\text{Chl a} = 12.25 \times (\text{A value of 663 nm}) - 2.79 \times (\text{A value of 647 nm}).$$

$$\text{Chl b} = 21.50 \times (\text{A value of 647 nm}) - 5.10 \times (\text{A value of 663 nm}).$$

$$\text{Car.} = 1000 \times (\text{A value of 470 nm}) - 1.82 \times (\text{Chlorophyll a}) - 95.15 \times (\text{Chlorophyll b}) / 225.$$

2.11. Relative water content (RWC; %)

At 60 days after sowing, RWC was assessed based on Barrs and Weatherley (1962) equation; $\text{RWC (\%)} = (\text{FM} - \text{DM}) / (\text{TM} - \text{DM}) \times 100$; where FM: fresh mass (g), TM: turgid mass (g), and DM is the dry mass (g).

2.12. Proline content ($\mu\text{mol g}^{-1}$ FW)

At 60 days after sowing, 5 mL of ethanol (95 %) was added to 0.5 g of leaf sample which was centrifuged at $5000 \times g$ according to procedure of Bates et al. (1973) using 2 mL of ninhydrin, and 2 mL of glacial acetic acid under water bath conditions (100 °C). Proline content was assessed at 520 nm by the UV-160A spectrophotometer (Shimadzu, Kyoto, Japan).

2.13. Antioxidant enzyme activity (SOD, POX, CAT, and APX; $\mu\text{mol mg}^{-1}$ protein min^{-1})

At 60 days after sowing, 0.5 g leaf sample was mixed with 5 mL of cold phosphate buffer (50 mM phosphate buffer pH 7.0, containing 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 % polyvinylpyrrolidone) and centrifuged at $15,000 \times g$, 4 °C for 30 min. Superoxide dismutase activity (SOD: 1.15.1.1) was estimated using 50 % NBT reduction at 560 nm according to Beauchamp and Fridovich (1971). Peroxidase activity (POX: 1.11.1.7) was estimated using o-phenylenediamine as a chromogenic assay

with H₂O₂ and enzyme extract at 417 nm according to Vetter et al. (1958)) Catalase activity (CAT: 1.11.1.6) was estimated using the reaction between 50 µL enzyme extract and 12.5 mm H₂O₂ with 50 mM K-phosphate buffer (pH 7.0) at 240 nm for 60 s according to Aebi (1984). Ascorbate peroxidase (APX) was estimated using 50 mg of PVP, 0.1 % Triton X-100, 10 % glycerol, 5 mM ascorbic acid and 1 mM EDTA. After 30 min at 4 °C, the homogenate was centrifuged at 18,000 g for 20 min at 4 °C. according to Jebara et al. (2005).

2.14. Hydrogen peroxide (H₂O₂; µmol g⁻¹ FW)

At 60 days after sowing, 0.5 g leaf sample, in the presence of liquid N₂, was mixed with hydro-acetone buffer (4:1 v/v), 0.65 % thiobarbituric acid (TBA) and 0.01 % butyl hydroxyl toluene (BHT) and shacked at 10,000 ×g for 15 min (Velikova et al., 2000). H₂O₂ was assessed at 390 nm by the UV-160A spectrophotometer (Shimadzu, Kyoto, Japan).

2.15. Lipid peroxidation (MDA; µmol g⁻¹ FW)

At 60 days after sowing, liquid N₂-frozen 0.5 g leaf sample was mixed with trichloroacetic acid (TCA: 0.1 %), and shacked at 3000 rpm for 20 min (Du and Bramlage 1992). MDA was assessed at 532 and 600 nm by the UV-160A spectrophotometer (Shimadzu, Kyoto, Japan).

2.16. Electrolyte leakage (EL; %)

At 60 days after sowing, 0.5 g leaf sample was mixed with 20 mL of distilled water in a test tube and shacked at 100 rpm at room temperature (Bajji et al., 2002). The EL % was assessed by the following formula:

$$EL (\%) = \frac{C1}{C2} \times 100$$

whereas C1 is the electrical conductivity (EC) after placing sample in a water bath at 60 °C for 1 h, and C2 is the EC after placing sample in a water bath at 100 °C for 10 min.

2.17. Yield-related parameters

At the harvest time, ten random plants were selected from each treatment and hand-threshed to measure the number of grains panicles⁻¹ and 1000 grain weight (g). Grain yield (ton ha⁻¹) was computed from a 8-m² area in each plot at 14 % of moisture content.

2.18. Statistical analysis

Data analysis was performed using Microsoft Excel 2016 and SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). A one-way ANOVA was conducted separately for the BC and PRGP treatments. Post-hoc comparisons were carried out using Tukey's test, with significance set at the p ≤ 0.05 level. Results are presented as mean ± standard deviation

Table 2

Effect of NaCl-derived salinity on the production of indole acetic acid (IAA) by six different PGPR strains and final germination percentage (FGP) of rice seeds.

PGPR strains	NaCl-derived salinity (dS m ⁻¹)				
	0	2	4	6	8
	IAA production (µg mL⁻¹)				
<i>Pseudomonas koreensis</i> MG209738	20.86 ± 0.26 a	17.38 ± 0.17 d	14.53 ± 0.26 h	11.57 ± 0.06 m	4.53 ± 0.26 p
<i>Azotobacter chroococcum</i> SARS 10	16.85 ± 0.10 e	13.37 ± 0.14 k	11.59 ± 0.12 m	9.17 ± 0.03 o	1.59 ± 0.12 r
<i>Azospirillum lipoferum</i> SP2	16.05 ± 0.17 f	13.61 ± 0.09 jk	11.16 ± 0.04 n	9.08 ± 0.02 o	1.16 ± 0.04 s
<i>Bacillus coagulans</i> NCAIM B 1086	18.70 ± 0.16c	16.63 ± 0.18 e	14.01 ± 0.01 i	11.24 ± 0.09 mn	4.01 ± 0.01 q
<i>Enterobacter cloacae</i> KX034162	15.69 ± 0.18 g	12.92 ± 0.07 l	10.95 ± 0.02 n	8.98 ± 0.02 o	0.95 ± 0.02 s
<i>Pseudomonas stutzeri</i>	19.96 ± 0.10 b	16.22 ± 0.08 f	13.87 ± 0.03 ij	10.97 ± 0.03 n	3.87 ± 0.03 q
	Final germination percentage (%)				
<i>Pseudomonas koreensis</i> MG209738	100 ± 0.0 a	100 ± 0.0 a	95.0 ± 0.0 b	75.0 ± 0.0 h	55.0 ± 0.0 l
<i>Azotobacter chroococcum</i> SARS 10	100 ± 0.0 a	100 ± 0.0 a	85.0 ± 0.0 e	56.0 ± 0.6 k	48.0 ± 0.0 m
<i>Azospirillum lipoferum</i> SP2	100 ± 0.0 a	100 ± 0.0 a	82.0 ± 0.0 f	59.0 ± 0.0 i	46.0 ± 0.0 n
<i>Bacillus coagulans</i> NCAIM B 1086	100 ± 0.0 a	100 ± 0.0 a	92.0 ± 0.0c	75.0 ± 0.0 h	55.0 ± 0.0 l
<i>Enterobacter cloacae</i> KX034162	100 ± 0.0 a	100 ± 0.0 a	78.3 ± 0.3 g	57.0 ± 0.0 j	41.0 ± 0.0 o
<i>Pseudomonas stutzeri</i>	100 ± 0.0 a	100 ± 0.0 a	90.00 ± 0.0 d	75.0 ± 0.0 h	55.0 ± 0.0 l

Data are mean ± SD with n = 3.

Means in the same column followed by different letters are significant according to the Tukey's test at p ≤ 0.05.

3. Results

3.1. Evaluation of PGPR strains

The evaluation of six PGPR strains under NaCl-derived salinity and cadmium (Cd) stress revealed significant differences in their ability to produce indole acetic acid (IAA) and support rice seed germination (Table 2 & 3). Under salinity stress (0–8 dS m⁻¹), *Pseudomonas koreensis* and *Pseudomonas stutzeri* exhibited the highest IAA production (20.86 and 19.96 µg mL⁻¹, respectively, at 0 dS m⁻¹), maintaining robust levels (11.57 and 10.97 µg mL⁻¹ at 6 dS m⁻¹), while *Bacillus coagulans* showed consistent performance (18.70 µg mL⁻¹ at 0 dS m⁻¹; 11.24 µg mL⁻¹ at 6 dS m⁻¹). These three strains also sustained high final germination percentages (FGP) under salinity (75–95 % at 6–8 dS m⁻¹), outperforming *Azotobacter chroococcum* and *Azospirillum lipoferum* (FGP: 41–58 %). Similarly, under Cd stress (0–20 mg L⁻¹), *P. koreensis*, *B. coagulans*, and *P. stutzeri* maintained elevated IAA levels (8.4–12.07 µg mL⁻¹ at 10 mg Cd L⁻¹) and germination rates (52–55 % at 20 mg Cd L⁻¹), whereas other strains declined sharply (IAA: 3.5–8.1 µg mL⁻¹; FGP: 39–44 %). The selection of *P. koreensis*, *B. coagulans*, and *P. stutzeri* for further studies was based on their superior stress resilience, demonstrated by sustained IAA production and germination rates under combined salinity and Cd stress, as well as their documented roles in heavy metal sequestration (*Pseudomonas* spp.) and nutrient mobilization (*B. coagulans*), which are critical for soil remediation and plant growth promotion in contaminated environments.

3.2. Response of soil physicochemical parameters to applied treatments under Cd and salinity stress

Across both seasons, treatments effectively reduced soil pH and EC compared to the control (CK). In 2022, soil pH was reduced from 8.50 in CK to 8.07 under the combined treatment, while EC decreased from 5.97 dS m⁻¹ to 4.39 dS m⁻¹. In 2023, similar reductions were observed, with the combined treatment lowering pH from 8.38 in CK to 8.02 and EC from 5.60 dS m⁻¹ to 4.01 dS m⁻¹ (Table 4).

SOM showed significant improvement under all treatments compared to CK in both seasons. In 2022, SOM increased from 1.07 % in CK to 1.14 % in the combined treatment, with identical values observed in 2023. CEC was markedly enhanced under the combined treatment, rising from 24.6 cmolc kg⁻¹ in CK to 29.1 cmolc kg⁻¹ in 2022 and further to 30.9 cmolc kg⁻¹ in 2023.

Both seasons demonstrated a consistent decrease in soil Na⁺ levels and ESP alongside an increase in K⁺ content under treatment. In 2022, Na⁺ levels were reduced from 27.68 meq L⁻¹ in CK to 25.37 meq L⁻¹ under the combined treatment, and ESP dropped from 19.0 % to 16.8 %. Similarly, in 2023, Na⁺ decreased to 25.12 meq L⁻¹ and ESP to 16.4 %. K⁺ levels improved from 0.31 meq L⁻¹ in CK to 0.43 meq L⁻¹ in 2022 and from 0.33 meq L⁻¹ to 0.45 meq L⁻¹ in 2023.

Improvements in soil physical properties were consistent between seasons. Soil bulk density was reduced significantly under the combined treatment, dropping from 1.49 g cm⁻³ in CK to 1.16 g cm⁻³ in 2022 and to 1.12 g cm⁻³ in 2023. Total porosity increased from 48.0 % in CK to 49.6 % in 2022 and to 49.7 % in 2023 under the combined treatment. Hydraulic conductivity demonstrated the most substantial improvement, rising from 2.90 cm day⁻¹ in CK to 3.93 cm day⁻¹ in 2022 and further to 4.38 cm day⁻¹ in 2023.

3.3. Response of soil microbiota to applied treatments under Cd and salinity stress

Soil respiration, a key indicator of microbial activity, was enhanced under all treatments compared to CK in both seasons (Fig. 1). In 2022, the highest CO₂ emission (43.05 mg CO₂ 100 g⁻¹ dry soil 24 h⁻¹) was observed in the combined treatment, significantly higher than CK (15.79 mg CO₂ 100 g⁻¹ dry soil 24 h⁻¹). A similar trend was observed in 2023, with the combined treatment showing the highest CO₂ emission (48.09 mg CO₂ 100 g⁻¹ dry soil 24 h⁻¹), confirming the results from 2022.

Table 3

Effect of different cadmium concentrations on the production of indole acetic acid (IAA) by six different PGPR strains and final germination percentage (FGP) of rice seeds.

PGPR strains	Cadmium concentrations (mg L ⁻¹)				
	0	5	10	15	20
	IAA production (µg mL⁻¹)				
<i>Pseudomonas koreensis</i> MG209738	16.50 ± 0.04c	15.17 ± 0.02 e	12.07 ± 0.02 h	8.40 ± 0.01 j	1.69 ± 0.46 m
<i>Azotobacter chroococcum</i> SARS 10	14.63 ± 0.19 f	11.96 ± 0.35 h	8.14 ± 0.04 jk	3.53 ± 0.02 l	0.80 ± 0.01 n
<i>Azospirillum lipoferum</i> SP2	15.80 ± 0.10 d	12.58 ± 0.06 g	8.01 ± 0.02 k	3.84 ± 0.01 l	0.78 ± 0.01 n
<i>Bacillus coagulans</i> NCAIM B 1086	16.95 ± 0.07 b	15.93 ± 0.04 d	11.54 ± 0.01 i	8.40 ± 0.00 j	2.03 ± 0.03 m
<i>Enterobacter cloacae</i> KX034162	15.21 ± 0.10 e	12.50 ± 0.08 g	8.15 ± 0.00 jk	3.89 ± 0.01 l	0.68 ± 0.06 n
<i>Pseudomonas stutzeri</i>	17.69 ± 0.16 a	15.92 ± 0.03 d	12.78 ± 0.05 g	8.41 ± 00 j	3.53 ± 0.02 l
	Final germination percentage (%)				
<i>Pseudomonas koreensis</i> MG209738	100 ± 0.0 a	100 ± 0.0 a	88.7 ± 0.9 c	74.3 ± 0.9 f	52.0 ± 0.6 k
<i>Azotobacter chroococcum</i> SARS 10	100 ± 0.0 a	100 ± 0.0 a	83.0 ± 0.6 d	58.3 ± 0.3 h	44.0 ± 0.6 l
<i>Azospirillum lipoferum</i> SP2	100 ± 0.0 a	100 ± 0.0 a	80.3 ± 0.3 e	54.0 ± 0.6 j	41.3 ± 0.3 m
<i>Bacillus coagulans</i> NCAIM B 1086	100 ± 0.0 a	100 ± 0.0 a	84.0 ± 0.6 d	72.0 ± 0.6 g	52.0 ± 0.6 k
<i>Enterobacter cloacae</i> KX034162	100 ± 0.0 a	100 ± 0.0 a	72.0 ± 0.6 g	54.0 ± 0.6 j	39.7 ± 0.3 n
<i>Pseudomonas stutzeri</i>	100 ± 0.0 a	100 ± 0.0 a	92.7 ± 0.9 b	73.0 ± 0.6 g	55.7 ± 0.3 i

Data are mean ± SD with n = 3.

Means in the same column followed by different letters are significant according to the Tukey's test at p ≤ 0.05.

Table 4

Response of soil physicochemical properties to treating rice (*Oryza sativa* L.) irrigated with cadmium-contaminated water (wastewater) in saline soil with selenium nanoparticles (SeNPs), PGPRs (*Pseudomonas koreensis* MG209738, *Bacillus coagulans* NCAIM B 1086, and *Pseudomonas stutzeri*), and their combination during two consecutive seasons (2022 and 2023). A negative control was applied (CK).

Year	Treatment	pH	EC _e [†] (dS m ⁻¹)	SOM [‡] (%)	CEC [§] (cmolc kg ⁻¹)	Na ⁺ (meq L ⁻¹)	K ⁺ (meq L ⁻¹)	ESP [‡] (%)	Soil bulk density (g cm ⁻³)	Total porosity (%)	Hydraulic conductivity (cm day ⁻¹)
2022	CK	8.50 ± 0.03a	5.97 ± 0.08a	1.07 ± 0.00d	24.6 ± 0.24	27.68 ± 0.30a	0.31 ± 0.01d	19.0 ± 0.18a	1.49 ± 0.03a	48.0 ± 0.01d	2.90 ± 0.04c
	SeNPs	8.36 ± 0.01b	5.43 ± 0.04b	1.09 ± 0.00c	25.2 ± 0.12	27.02 ± 0.02b	0.36 ± 0.01c	18.4 ± 0.08b	1.42 ± 0.00b	48.2 ± 0.01c	3.21 ± 0.09b
	PGPRs	8.28 ± 0.01c	4.68 ± 0.04c	1.11 ± 0.00b	28.3 ± 0.18	26.05 ± 0.02c	0.40 ± 0.01b	17.8 ± 0.11c	1.23 ± 0.00c	49.4 ± 0.01b	3.82 ± 0.05a
	Combination	8.07 ± 0.01d	4.39 ± 0.02d	1.14 ± 0.00a	29.1 ± 0.53	25.37 ± 0.15d	0.43 ± 0.01a	16.8 ± 0.45d	1.16 ± 0.01d	49.6 ± 0.01a	3.93 ± 0.05a
2023	CK	8.38 ± 0.01a	5.60 ± 0.01a	1.07 ± 0.00c	25.4 ± 0.27	27.52 ± 0.09a	0.33 ± 0.01d	18.6 ± 0.18a	1.47 ± 0.01a	48.0 ± 0.01d	3.20 ± 0.06d
	SeNPs	8.32 ± 0.01b	5.03 ± 0.04b	1.11 ± 0.00b	26.0 ± 0.34	26.56 ± 0.20b	0.39 ± 0.01c	18.2 ± 0.11b	1.38 ± 0.02b	48.4 ± 0.01c	3.36 ± 0.05c
	PGPRs	8.29 ± 0.01c	4.80 ± 0.02c	1.14 ± 0.03a	28.7 ± 0.12	25.73 ± 0.04c	0.42 ± 0.01b	17.3 ± 0.15c	1.20 ± 0.00c	49.3 ± 0.03b	3.88 ± 0.03b
	Combination	8.02 ± 0.01d	4.01 ± 0.06d	1.14 ± 0.00a	30.9 ± 0.08	25.12 ± 0.04d	0.45 ± 0.01a	16.4 ± 0.10d	1.12 ± 0.01d	49.7 ± 0.01a	4.38 ± 0.06a

[†] Electrical conductivity of soil paste extract;

[‡] Soil organic matter;

[§] Cation exchange capacity;

[‡] exchangeable sodium percentage.

Means in the same column of the same year followed by different letters are significant according to the Tukey's test at $p \leq 0.05$. Data are mean ± SD. n = 3.

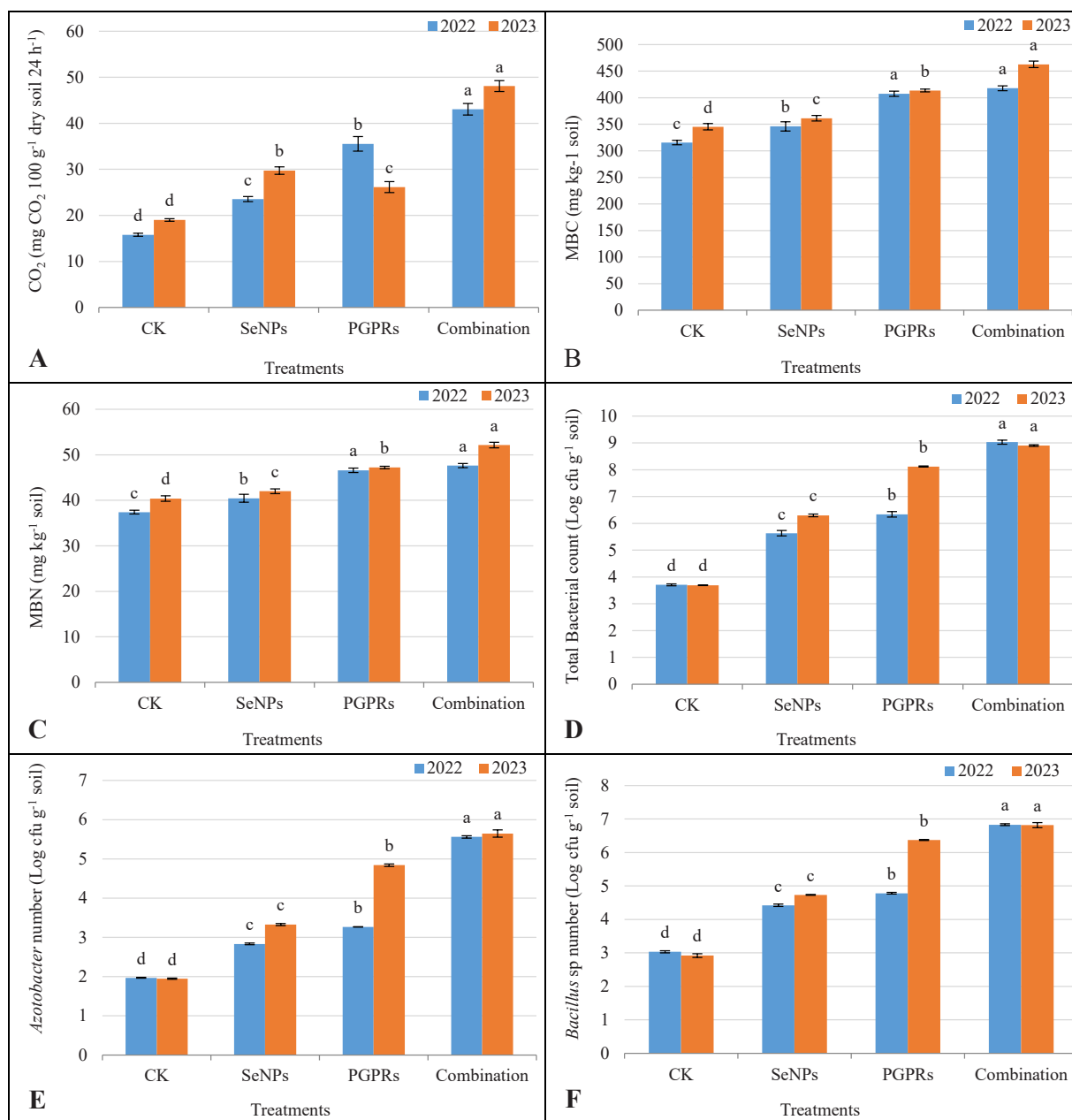


Fig. 1. Response of (A) soil respiration, (B) microbial biomass carbon, (C) microbial biomass nitrogen, (D) total bacterial count, (E) *Azotobacter* number, and (F) *Bacillus* sp. number of saline soil cultivated with rice (*Oryza sativa* L.) and irrigated with cadmium-contaminated water (waste-water) to selenium nanoparticles (SeNPs), PGPRs (*Pseudomonas koreensis* MG209738, *Bacillus coagulans* NCAIM B 1086, and *Pseudomonas stutzeri*), and their combination during two consecutive seasons (2022 and 2023). A negative control was applied (CK). Different letters on bars of the same year are significant according to the Tukey's test at $p \leq 0.05$. Data are mean \pm SD. $n = 3$.

The treatments consistently improved MBC and MBN in both seasons. In 2022, MBC increased from 315.58 mg kg⁻¹ soil in CK to 417.75 mg kg⁻¹ in the combined treatment, while MBN rose from 37.40 mg kg⁻¹ soil to 47.62 mg kg⁻¹. In 2023, these improvements were further validated, with MBC increasing to 462.85 mg kg⁻¹ and MBN to 52.13 mg kg⁻¹ under the combined treatment, underscoring the reproducibility of these results.

The abundance of total bacteria, *Azotobacter*, and *Bacillus* sp. increased significantly under all treatments compared to CK. In 2022, the combined treatment resulted in the highest bacterial count (Log 9.03 cfu g⁻¹ soil), *Azotobacter* (Log 5.56 cfu g⁻¹ soil), and *Bacillus* sp. (Log 6.83 cfu g⁻¹ soil). These results were consistent in 2023, with slightly higher counts of total bacteria (Log 8.90 cfu g⁻¹ soil), *Azotobacter* (Log 5.65 cfu g⁻¹ soil), and *Bacillus* sp. (Log 6.82 cfu g⁻¹ soil) under the combined treatment.

3.4. Response of soil enzyme activity to applied treatments under Cd and salinity stress

Acid phosphatase activity, an indicator of phosphorus availability, was consistently improved by the treatments (Fig. 2). In 2022, the combined treatment increased acid phosphatase activity from 2.88 mg phenol $\text{kg}^{-1} \text{day}^{-1}$ in CK to 3.77 mg phenol $\text{kg}^{-1} \text{day}^{-1}$. In 2023, acid phosphatase activity reached 3.96 mg phenol $\text{kg}^{-1} \text{day}^{-1}$ under the combined treatment, confirming the previous season's findings.

Urease activity, crucial for nitrogen cycling, showed consistent improvements under all treatments. In 2022, urease activity increased from 0.34 mg $\text{NH}_4^+\text{-N g}^{-1} \text{day}^{-1}$ in CK to 0.45 mg $\text{NH}_4^+\text{-N g}^{-1} \text{day}^{-1}$ under the combined treatment. Similar results were observed in 2023, with urease activity increasing from 0.36 mg $\text{NH}_4^+\text{-N g}^{-1} \text{day}^{-1}$ in CK to 0.48 mg $\text{NH}_4^+\text{-N g}^{-1} \text{day}^{-1}$ under the combined treatment.

Catalase activity, an indicator of oxidative stress mitigation, was consistently higher under all treatments compared to CK. In 2022, catalase activity increased from 0.61 mg $\text{KMnO}_4 \text{g}^{-1} \text{day}^{-1}$ in CK to 0.91 mg $\text{KMnO}_4 \text{g}^{-1} \text{day}^{-1}$ under the combined treatment. Similarly, in 2023, catalase activity was highest in the combined treatment (0.99 mg $\text{KMnO}_4 \text{g}^{-1} \text{day}^{-1}$), confirming the trends observed in 2022.

Invertase activity, reflecting carbon cycling potential, showed a modest increase across all treatments. In 2022, invertase activity rose from 168.08 mg glucose $\text{g}^{-1} \text{day}^{-1}$ in CK to 171.13 mg glucose $\text{g}^{-1} \text{day}^{-1}$ under the combined treatment. In 2023, the same trend was observed, with invertase activity increasing from 168.49 mg glucose $\text{g}^{-1} \text{day}^{-1}$ in CK to 172.10 mg glucose $\text{g}^{-1} \text{day}^{-1}$ under the combined treatment.

3.5. Residual Cd in soil and accumulation in plant tissues after treatments

In both growing seasons, the combined treatment (SeNPs + PGPRs) significantly reduced soil Cd content compared to CK (Fig. 3). In 2022, soil Cd was reduced by 34.5 % (from 0.87 mg kg^{-1} in CK to 0.57 mg kg^{-1} in the combined treatment). Similarly, in 2023, the reduction reached 34.6 % (from 0.81 mg kg^{-1} in CK to 0.53 mg kg^{-1} in the combined treatment). These reductions were consistent

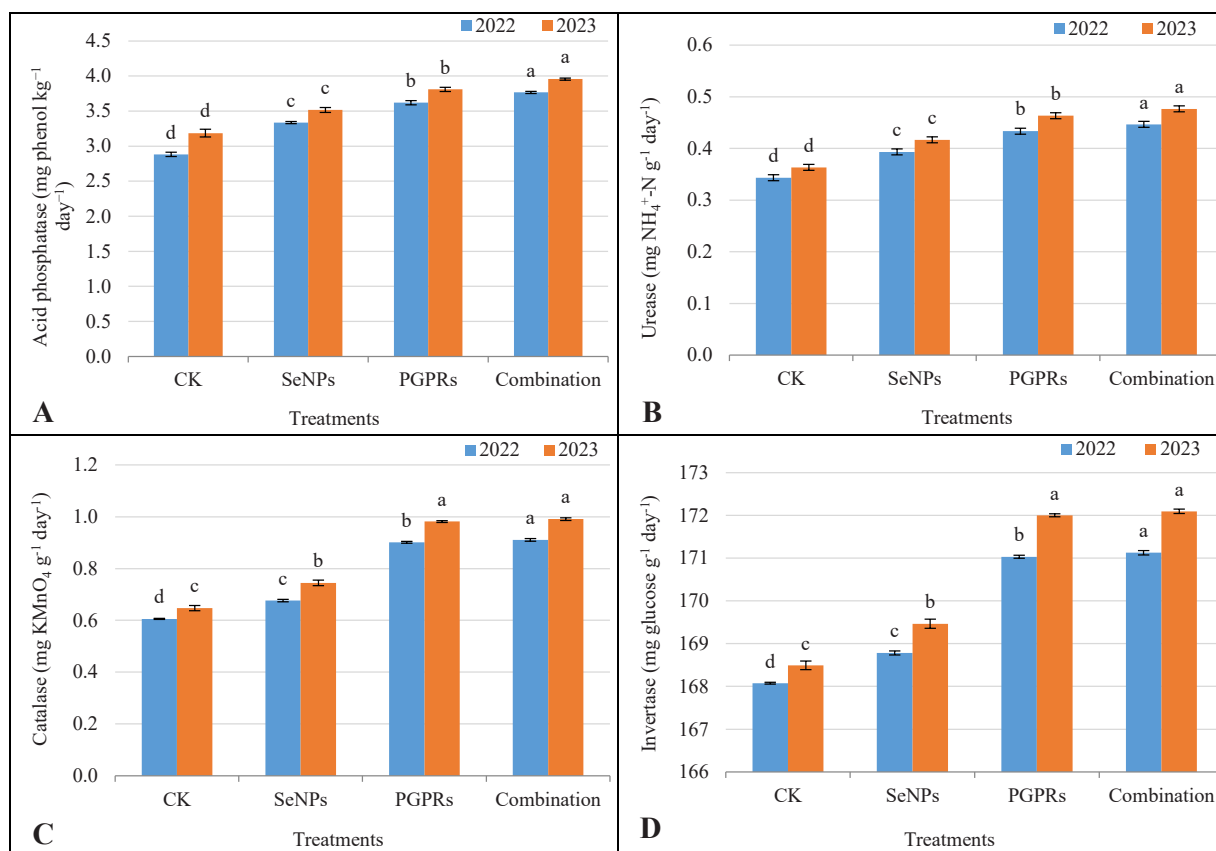


Fig. 2. Response of soil enzymes (A) acid phosphatase, (B) urease, (C) catalase, and (D) invertase to irrigation rice (*Oryza sativa* L.) plants with cadmium-contaminated water (wastewater) in saline soil and to application of selenium nanoparticles (SeNPs), PGPRs (*Pseudomonas koreensis* MG209738, *Bacillus coagulans* NCAIM B 1086, and *Pseudomonas stutzeri*), and their combination during two consecutive seasons (2022 and 2023). A negative control was applied (CK). Different letters on bars of the same year are significant according to the Tukey's test at $p \leq 0.05$. Data are mean \pm SD. $n = 3$.

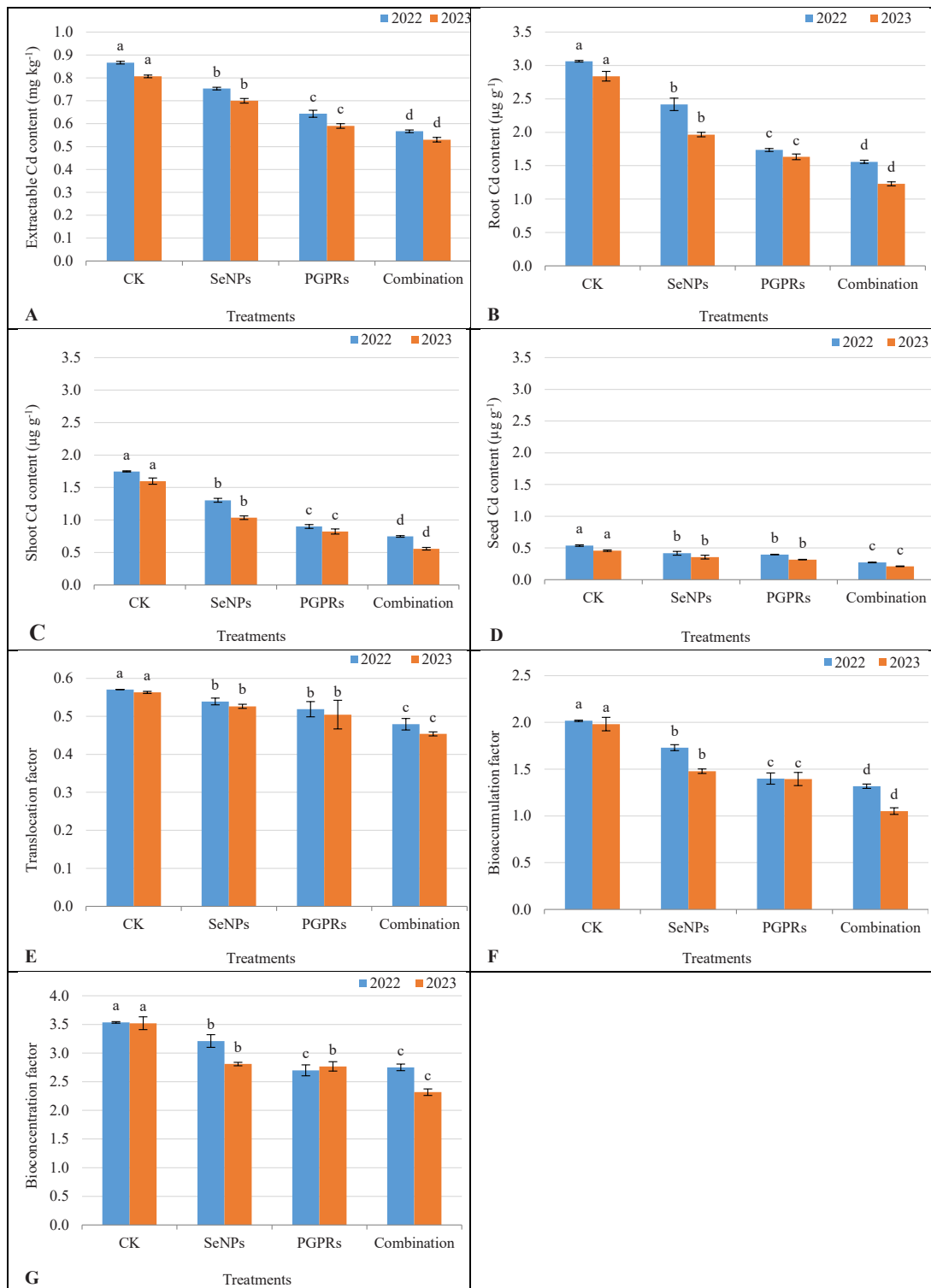


Fig. 3. Response of (A) soil extractable cadmium, (B) root cadmium content, (C) shoot cadmium content, (D) seed cadmium content, (E) translocation factor (TF), (F) bioaccumulation factor (BAF), and (G) bioconcentration factor (BCF) to irrigation rice (*Oryza sativa* L.) plants with cadmium-contaminated water (wastewater) in saline soil and to application of selenium nanoparticles (SeNPs), PGPRs (*Pseudomonas koreensis* MG209738, *Bacillus coagulans* NCAIM B 1086, and *Pseudomonas stutzeri*), and their combination during two consecutive seasons (2022 and 2023). A negative control was applied (CK). Different letters on bars of the same year are significant according to the Tukey's test at $p \leq 0.05$. Data are mean \pm SD. $n = 3$.

across both years, affirming the efficacy of the combined treatment in mitigating soil Cd contamination.

Cd accumulation in roots and shoots followed a similar trend, with the combined treatment showing the lowest Cd concentrations. In 2022, root Cd was reduced from $3.06 \mu\text{g g}^{-1}$ in CK to $1.56 \mu\text{g g}^{-1}$ under the combined treatment, while shoot Cd decreased from $1.75 \mu\text{g g}^{-1}$ in CK to $0.75 \mu\text{g g}^{-1}$. Corresponding reductions in 2023 were from $2.84 \mu\text{g g}^{-1}$ to $1.23 \mu\text{g g}^{-1}$ in roots and from $1.60 \mu\text{g g}^{-1}$ to $0.56 \mu\text{g g}^{-1}$ in shoots.

The Cd content in seeds was significantly reduced by all treatments compared to CK. In 2022, the Cd content decreased from $0.54 \mu\text{g g}^{-1}$ in CK to $0.42 \mu\text{g g}^{-1}$ with SeNPs, $0.40 \mu\text{g g}^{-1}$ with PGPR, and $0.27 \mu\text{g g}^{-1}$ with the combined treatment. A similar trend was observed in 2023, where the Cd content decreased from $0.46 \mu\text{g g}^{-1}$ in CK to $0.36 \mu\text{g g}^{-1}$ with SeNPs, $0.32 \mu\text{g g}^{-1}$ with PGPR, and $0.21 \mu\text{g g}^{-1}$ with the combined treatment. The combined treatment showed the most pronounced reduction in Cd content in both years.

The combined treatment consistently reduced the BCF, TF, and BAC in both years, indicating reduced Cd mobility and bioavailability within the plant system. For instance, BCF values in 2022 decreased from 3.54 in CK to 2.75 in the combined treatment, with similar reductions observed in 2023 (3.52 in CK to 2.32 in the combined treatment). TF values followed a comparable pattern, declining from 0.57 in CK to 0.48 (2022) and 0.56–0.45 (2023). BAC values exhibited the most substantial decrease under the combined treatment, dropping from 2.02 to 1.32 (2022) and 1.98–1.05 (2023).

3.6. Accumulation of selenium in plant tissues

In both years, the application of SeNPs and the combined treatment significantly increased the Se content in rice shoots and seeds compared to CK (Fig. 4). In 2022, the Se content in leaves increased from $0.55 \mu\text{g g}^{-1}$ in CK to $1.08 \mu\text{g g}^{-1}$ with SeNPs and further to $1.35 \mu\text{g g}^{-1}$ with the combined treatment. Similarly, in 2023, the Se content in shoot rose from $0.71 \mu\text{g g}^{-1}$ in CK to $1.15 \mu\text{g g}^{-1}$ with SeNPs and $1.47 \mu\text{g g}^{-1}$ with the combined treatment. The same trend was observed in seeds, where the Se content increased from $0.01 \mu\text{g g}^{-1}$ in CK to $0.33 \mu\text{g g}^{-1}$ with SeNPs and $0.49 \mu\text{g g}^{-1}$ with the combined treatment in 2022. In 2023, the Se content in seeds increased from $0.02 \mu\text{g g}^{-1}$ in CK to $0.34 \mu\text{g g}^{-1}$ with SeNPs and $0.51 \mu\text{g g}^{-1}$ with the combined treatment. The PGPR treatment alone also enhanced Se content, but to a lesser extent compared to SeNPs and the combined treatment.

3.7. Response of osmolytes and photosynthetic pigments of rice to applied treatments under Cd and salinity stress

Across both seasons, the combined treatment (SeNPs + PGPRs) significantly reduced plant Na^+ content and increased K^+ content compared to CK (Fig. 5). In 2022, Na^+ content decreased from 4.29mg g^{-1} in CK to 2.39mg g^{-1} under the combined treatment, while K^+ content increased from 0.34mg g^{-1} to 2.13mg g^{-1} . A similar trend was observed in 2023, with Na^+ content reducing from 3.51mg g^{-1} to 1.85mg g^{-1} and K^+ content increasing from 1.25mg g^{-1} to 2.83mg g^{-1} . These changes resulted in a markedly improved K^+/Na^+ ratio under the combined treatment, rising from 0.08 (2022 CK) to 0.89 (2022 combined) and from 0.36 (2023 CK) to 1.53 (2023 combined). The data from 2023 closely align with the findings from 2022, demonstrating consistency and reliability.

Pigment contents (chlorophyll a, chlorophyll b, and carotenoids) were significantly enhanced under the combined treatment. In 2022, chlorophyll a under the combined treatment increased to 1.62mg g^{-1} compared to 0.81mg g^{-1} in CK, with similar gains in chlorophyll b (0.17mg g^{-1} in CK vs. 0.94mg g^{-1}), and carotenoids (0.22mg g^{-1} in CK vs. 0.62mg g^{-1}). Pigment concentrations also exhibited consistent improvements, with chlorophyll a increasing to 1.72mg g^{-1} , chlorophyll b to 1.04mg g^{-1} , and carotenoids to 0.69mg g^{-1} under the combined treatment.

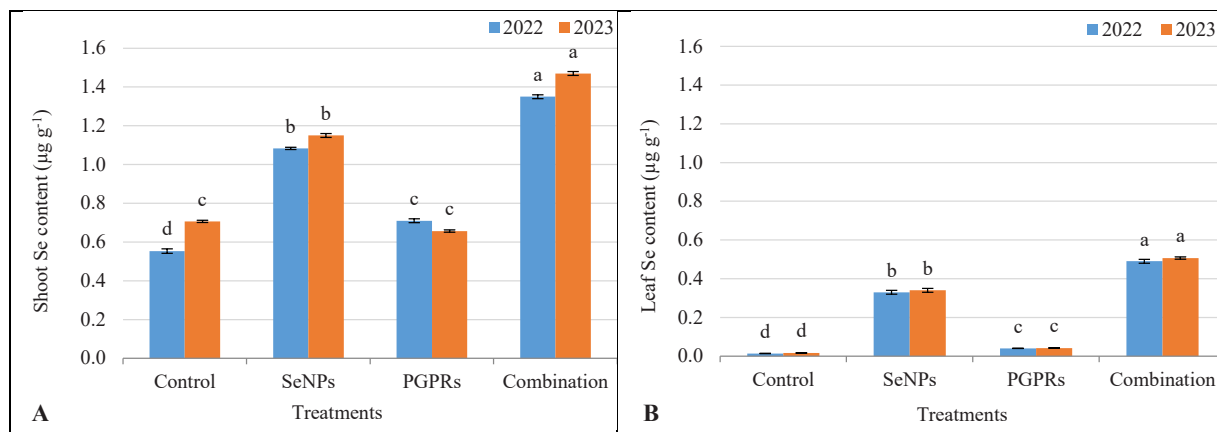


Fig. 4. Building up of selenium in (A) shoot and (B) seeds of rice (*Oryza sativa* L.) plants irrigated with cadmium-contaminated water (wastewater) in saline soil and to application of selenium nanoparticles (SeNPs), PGPRs (*Pseudomonas koreensis* MG209738, *Bacillus coagulans* NCAIM B 1086, and *Pseudomonas stutzeri*), and their combination during two consecutive seasons (2022 and 2023). A negative control was applied (CK). Different letters on bars of the same year are significant according to the Tukey's test at $p \leq 0.05$. Data are mean \pm SD. n = 3.

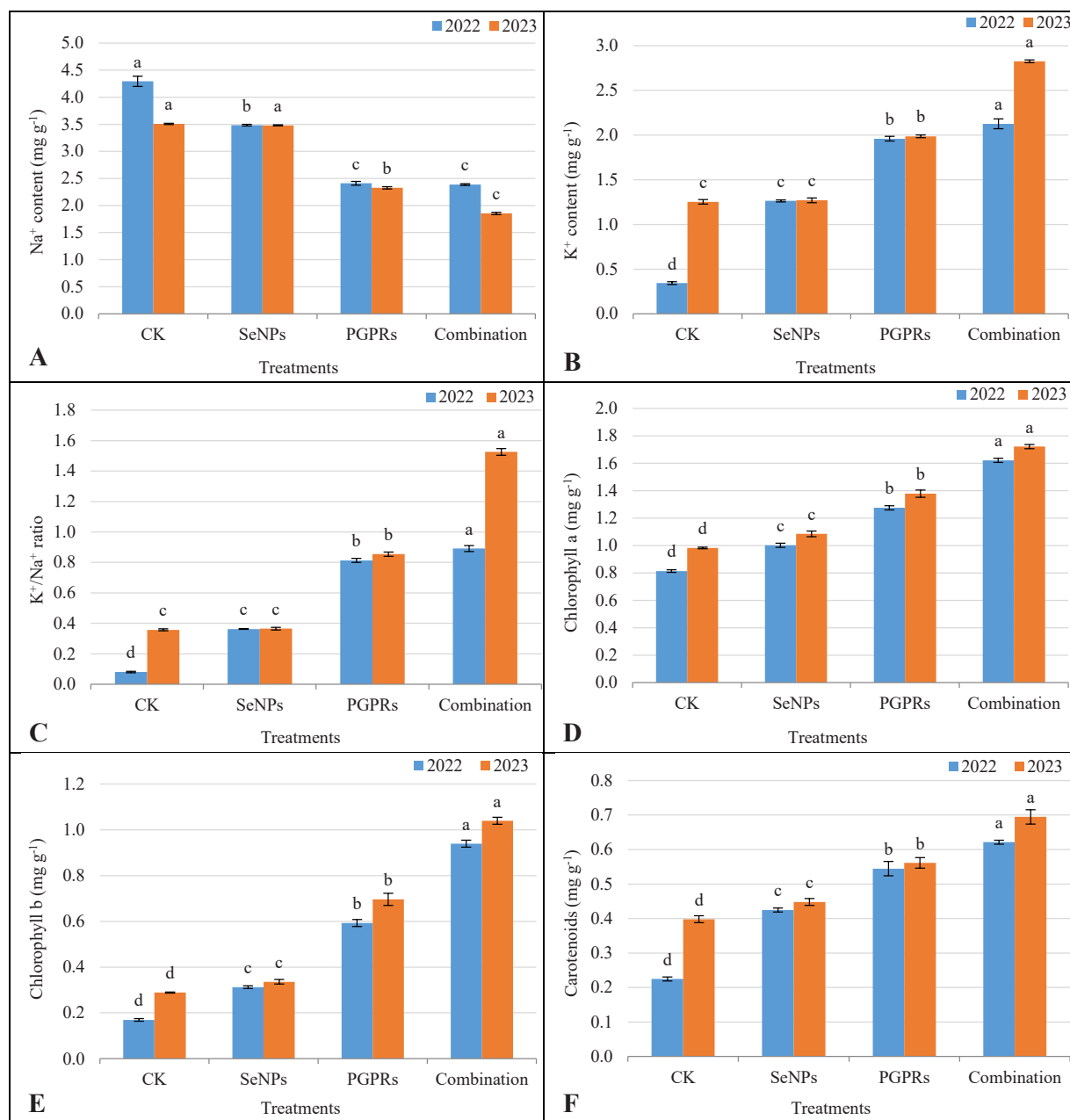


Fig. 5. Response of (A) Na⁺, (B) K⁺, (C) K⁺/Na⁺, (D) chlorophyll a, (E) chlorophyll b, and (F) carotenoid contents to irrigation rice (*Oryza sativa* L.) plants with cadmium-contaminated water (wastewater) in saline soil and to application of selenium nanoparticles (SeNPs), PGPRs (*Pseudomonas koreensis* MG209738, *Bacillus coagulans* NCAIM B 1086, and *Pseudomonas stutzeri*), and their combination during two consecutive seasons (2022 and 2023). A negative control was applied (CK). Different letters on bars of the same year are significant according to the Tukey's test at p ≤ 0.05. Data are mean ± SD. n = 3.

3.8. Response of antioxidant enzymes of rice applied treatments under Cd and salinity stress

SOD activity significantly increased across all treatments compared to CK in both seasons (Fig. 6). The combined treatment of SeNPs and microbial inoculation recorded the highest SOD activity, reaching 80.96 μmol mg⁻¹ protein min⁻¹ in 2022 and 86.21 μmol mg⁻¹ protein min⁻¹ in 2023. These values represent increases of 101.9 % (2022) and 88.7 % (2023) compared to CK. The results from 2023 corroborate the observations from 2022, affirming the consistent effectiveness of the combined treatment.

POX activity was also elevated under all treatments relative to CK, with the combined treatment achieving the highest activity (1.01 μmol mg⁻¹ protein min⁻¹ in 2022 and 1.05 μmol mg⁻¹ protein min⁻¹ in 2023). While increases in POX activity were moderate compared

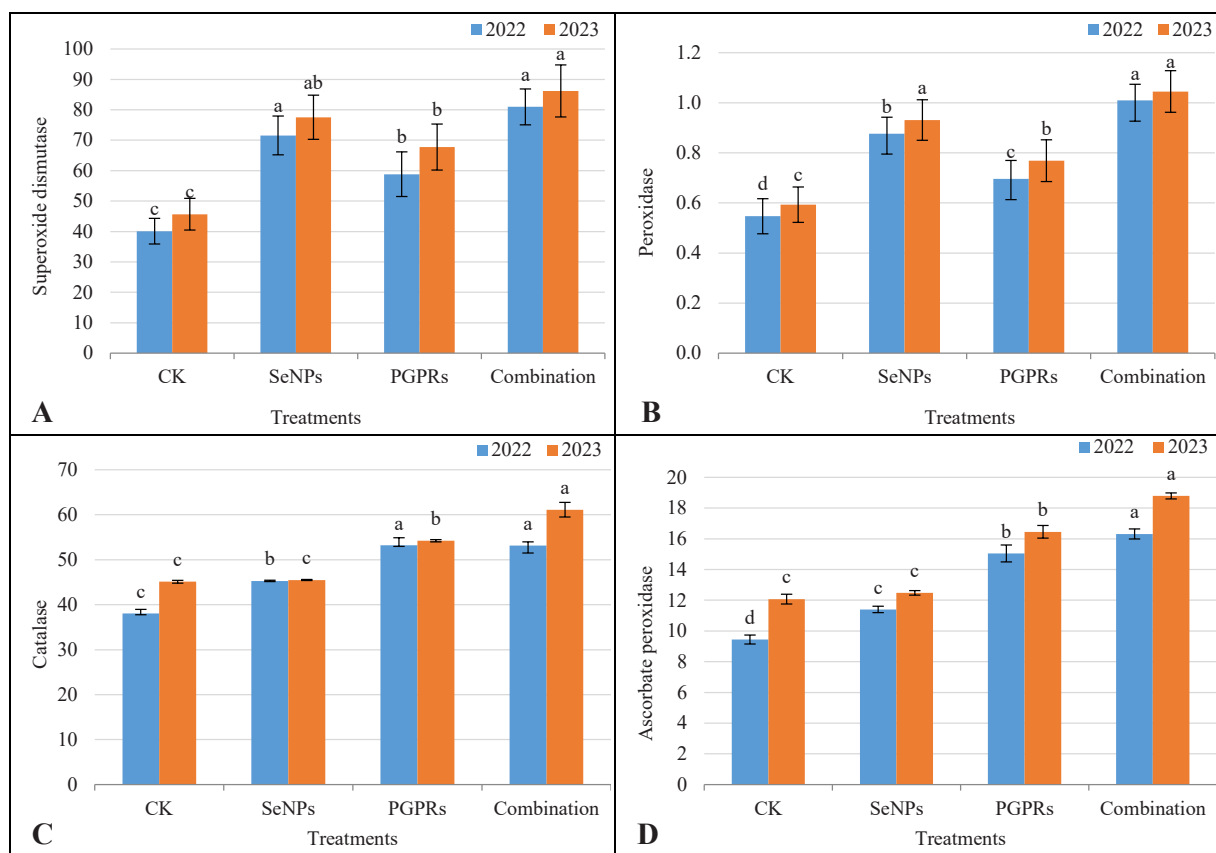


Fig. 6. Response of antioxidant enzymes ($\mu\text{mol mg}^{-1} \text{protein min}^{-1}$) (A) superoxide dismutase, (B) peroxidase, (C) catalase, and (D) ascorbate peroxidase to irrigation rice (*Oryza sativa* L.) plants with cadmium-contaminated water (wastewater) in saline soil and to application of selenium nanoparticles (SeNPs), PGPRs (*Pseudomonas koreensis* MG209738, *Bacillus coagulans* NCAIM B 1086, and *Pseudomonas stutzeri*), and their combination during two consecutive seasons (2022 and 2023). A negative control was applied (CK). Different letters on bars of the same year are significant according to the Tukey's test at $p \leq 0.05$. Data are mean \pm SD. $n = 3$.

to other enzymes, the combined treatment consistently outperformed single treatments and CK, showing reproducibility across the two years.

CAT activity showed marked improvements under the combined treatment, rising from $38.08 \mu\text{mol mg}^{-1} \text{protein min}^{-1}$ in CK to $53.12 \mu\text{mol mg}^{-1} \text{protein min}^{-1}$ in 2022 and further increasing to $61.13 \mu\text{mol mg}^{-1} \text{protein min}^{-1}$ in 2023. These results confirm the significant role of the combined treatment in enhancing oxidative stress mitigation, as evidenced by higher CAT activity in both years.

APX activity exhibited the greatest enhancement among all measured enzymes. In 2022, APX activity increased from $9.44 \mu\text{mol mg}^{-1} \text{protein min}^{-1}$ in CK to $16.32 \mu\text{mol mg}^{-1} \text{protein min}^{-1}$ under the combined treatment. A similar increase was observed in 2023, where APX activity rose from $12.07 \mu\text{mol mg}^{-1} \text{protein min}^{-1}$ in CK to $18.79 \mu\text{mol mg}^{-1} \text{protein min}^{-1}$ under the combined treatment. These findings highlight the efficacy of the combined treatment in promoting robust enzymatic defenses against oxidative stress.

3.9. Response of oxidative stress and cell membrane stability indicators of rice applied treatments under Cd and salinity stress

The MDA and H_2O_2 levels, key markers of oxidative stress, were significantly reduced in all treatments compared to CK in both 2022 and 2023 (Fig. 7). In 2022, MDA levels were reduced by 22.6 %, 61.4 %, and 76.7 % in the SeNPs, microbial inoculation, and combined treatments, respectively, compared to CK. The trend was similar in 2023, with reductions of 12.1 %, 63.3 %, and 90.4 % for the same treatments. Likewise, H_2O_2 content followed a comparable pattern, showing reductions of 16.5 %, 44.8 %, and 63.2 % in 2022 and 7.8 %, 40.9 %, and 67.7 % in 2023.

EL, an indicator of membrane stability, was substantially lower in treated plants. In 2022, the combined treatment resulted in a remarkable 66.4 % decrease in EL compared to CK. This effect was confirmed in 2023, with a similar reduction of 80.3 %, demonstrating consistent improvement in membrane stability.

Proline content, an osmoprotectant, decreased significantly in treated plants compared to CK, indicating reduced stress. In 2022, proline levels decreased by 31.8 %, 27.6 %, and 50.1 % in the SeNPs, microbial inoculation, and combined treatments, respectively. The combined treatment also resulted in the lowest proline accumulation in 2023 (47.5 % reduction compared to CK).

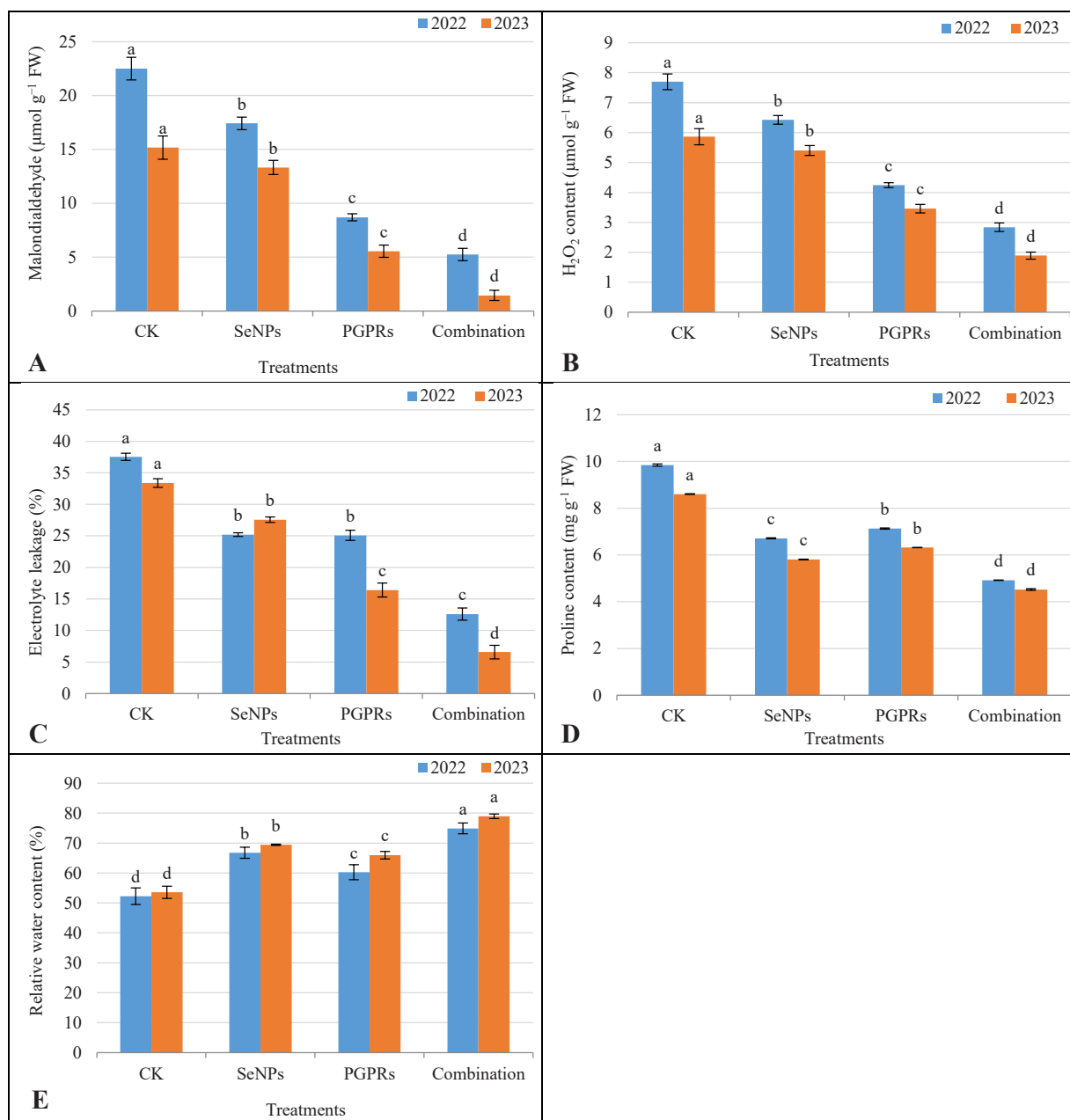


Fig. 7. Variations in (A) malondialdehyde content, (B) H_2O_2 content, (C) electrolyte leakage, (D) proline content, and (E) relative water content of rice (*Oryza sativa* L.) plants irrigated with cadmium-contaminated water (wastewater) in saline soil and treated with selenium nanoparticles (SeNPs), PGPRs (*Pseudomonas koreensis* MG209738, *Bacillus coagulans* NCAIM B 1086, and *Pseudomonas stutzeri*), and their combination during two consecutive seasons (2022 and 2023). A negative control was applied (CK). Different letters on bars of the same year are significant according to the Tukey's test at $p \leq 0.05$. Data are mean \pm SD. $n = 3$.

RWC, a measure of plant water status, increased markedly in treated plants. The combined treatment resulted in the highest RWC values in both years, with increases of 43.3 % in 2022 and 47.5 % in 2023 relative to CK. This trend indicates that the treatments effectively mitigated drought and salinity stress, promoting improved water retention.

3.10. Response of yield of rice applied treatments under Cd and salinity stress

The treatments had a significant impact on grain production parameters in both 2022 and 2023 (Table 5). The 1000-grain weight, number of grains per panicle, and grain yield showed consistent improvements in the treated plants compared to CK across both

Table 5

Response of yield and yield components of rice (*Oryza sativa* L.) irrigated with cadmium-contaminated water (wastewater) in saline soil to selenium nanoparticles (SeNPs), PGPRs (*Pseudomonas koreensis* MG209738, *Bacillus coagulans* NCAIM B 1086, and *Pseudomonas stutzeri*), and their combination during two consecutive seasons (2022 and 2023). A negative control was applied (CK).

Treatment	Leaf area (cm ²)		1000-grain weight (g)		No. grains (panicle ⁻¹)		Grain yield (ton ha ⁻¹)	
	2022	2023	2022	2023	2022	2023	2022	2023
CK	2.60 ± 0.02d	3.51 ± 0.03c	18.6 ± 0.33c	19.3 ± 0.22d	104 ± 1.4c	107 ± 1.6d	5.8 ± 0.08c	5.8 ± 0.10c
SeNPs	3.52 ± 0.01c	3.53 ± 0.03c	20.1 ± 0.18b	20.7 ± 0.58c	108 ± 1.5b	111 ± 1.3c	6.6 ± 0.24b	6.7 ± 0.09b
PGPRs	4.22 ± 0.03b	4.25 ± 0.02b	21.1 ± 0.17b	21.8 ± 0.47b	110 ± 1.6b	113 ± 0.4b	6.7 ± 0.11b	6.8 ± 0.13b
Combination	4.39 ± 0.06a	4.49 ± 0.02a	22.4 ± 1.02a	23.4 ± 0.56a	115 ± 1.7a	117 ± 0.2a	7.1 ± 0.12a	7.2 ± 0.09a

Means in the same column followed by different letters are significant according to the Tukey's test at $p \leq 0.05$. Data are mean ± SD. n = 10 for the first three parameters.

seasons. The leaf area under the combined treatment increased to 4.39 cm² compared to 2.60 cm² in CK. In 2023, the leaf area further increased under the combined treatment to 4.49 cm² (compared to 3.51 cm² in CK).

In 2022, the 1000-grain weight increased from 18.62 g in the control to 20.15 g, 21.09 g, and 22.40 g in the Bio-SeNPs, microbial inoculation, and combined treatments, respectively. A similar trend was observed in 2023, with the combined treatment achieving the highest weight of 23.38 g, a 21.4 % increase compared to CK.

The combined treatment consistently produced the highest number of grains per panicle, increasing from 104.10 in CK to 115.04 in 2022 and from 107.28 in CK to 117.10 in 2023. Both microbial inoculation and SeNPs treatments also improved panicle productivity compared to CK, with results closely matching those of the combined treatment.

Grain yield showed notable enhancement in response to the treatments. In 2022, the grain yield improved from 5.76 ton ha⁻¹ in CK to 6.55, 6.65, and 7.06 ton ha⁻¹ for SeNPs, microbial inoculation, and combined treatments, respectively. In 2023, the combined treatment resulted in a yield of 7.24 ton ha⁻¹, a 25.0 % increase over CK, confirming the findings from 2022.

4. Discussion

4.1. Variations in soil physicochemical properties

The results of this study demonstrate that the combined treatment of SeNPs and PGPRs consistently reduced soil pH and EC_e across both seasons, indicating a mitigation of salinity stress. Similar findings were reported by Alharbi et al. (2022a), who observed that the application of potassium humate and PGPRs reduced soil EC_e and improved soil structure in salt-affected soils. The reduction in Na⁺ levels and ESP in our study aligns with the results of Nehela et al. (2021), who demonstrated that biochar and PGPRs synergistically reduced Na⁺ accumulation and improved K⁺/Na⁺ ratios in saline soils. The increase in SOM and CEC under our treatments is consistent with the findings of (Hafez et al. (2019)), who reported that biochar and PGPRs enhanced SOM and CEC, thereby improving soil fertility and nutrient retention. The improvement in soil physical properties, such as reduced bulk density and increased hydraulic conductivity, can be attributed to the synergistic effects of SeNPs and PGPRs. Alharbi et al. (2022b) found that silica nanoparticles (SiNPs) combined with PGPRs improved soil porosity and water infiltration in salt-affected soils, which supports our observations. The enhanced hydraulic conductivity in our study is particularly significant, as it indicates improved water movement and reduced waterlogging, which are critical for plant growth in saline and Cd-contaminated soils.

The reduction in soil salinity and Cd stress can be explained by several mechanisms. First, SeNPs have been shown to mitigate heavy metal toxicity by reducing metal uptake and enhancing antioxidant defense systems in plants (Feng et al., 2021). In our study, the application of SeNPs likely reduced Cd bioavailability in the soil, as evidenced by the improved soil properties and plant growth. Zhu et al. (2022) also reported that foliar application of biosynthetic SeNPs alleviated Cd, Pb, and Hg toxicity in *Brassica chinensis* by inhibiting heavy metal adsorption and improving the plant's antioxidant system. Second, PGPRs play a crucial role in alleviating salinity and heavy metal stress by producing exopolysaccharides, siderophores, and phytohormones, which enhance nutrient uptake and stress tolerance in plants (Alshaal et al., 2024). The inoculation of PGPRs in our study likely improved plant resilience to salinity and Cd stress by enhancing root growth and nutrient acquisition, as well as by reducing Na⁺ accumulation and improving K⁺/Na⁺ ratios. This is consistent with the findings of Omara et al. (2022), who reported that PGPRs improved wheat growth and yield under deficit irrigation in salt-affected soils.

The combined treatment of SeNPs and PGPRs exhibited the most significant improvements in soil and plant properties, suggesting a synergistic effect. This is in line with the findings of Shang et al. (2024), who demonstrated that biosynthesized SeNPs combined with PGPRs effectively combated soil metal stresses in rice by enhancing soil health and plant antioxidant capacity. The synergistic effect likely arises from the complementary mechanisms of SeNPs and PGPRs, where SeNPs reduce metal toxicity and oxidative stress, while PGPRs enhance nutrient availability and plant growth.

4.2. Changes in soil microbial activity and biomass after SeNPs and PGPRs application

The significant increase in soil respiration under all treatments, particularly the combined treatment, indicates enhanced microbial activity. This is consistent with the findings of Kheyrodin et al. (2012), who reported that organic amendments and microbial

inoculants increased soil respiration and microbial biomass in stressed soils. The higher CO₂ emissions observed in our study suggest that the treatments stimulated microbial metabolism, likely due to the improved availability of organic carbon and nutrients. Similarly, Hafez et al. (2019) found that biochar and PGPRs synergistically enhanced soil respiration and microbial activity in salt-affected soils, supporting our results.

The increase in MBC and MBN under the treatments further confirms the positive impact on soil microbial communities. The combined treatment showed the highest MBC and MBN values, indicating a synergistic effect of SeNPs and PGPRs. This aligns with the findings of Alharbi et al. (2022b), who demonstrated that SiNPs combined with PGPRs increased microbial biomass in saline soils. The enhanced microbial biomass likely contributed to improved nutrient cycling and soil fertility, which are essential for plant growth under stress conditions.

The treatments significantly increased the abundance of total bacteria, *Azotobacter*, and *Bacillus* sp., indicating a shift toward a more beneficial microbial community. The combined treatment resulted in the highest bacterial counts, suggesting that SeNPs and PGPRs together created a more favorable environment for microbial growth. This is consistent with the findings of Thongnok et al. (2022), who reported that heavy metal-resistant PGPRs enhanced the abundance of beneficial bacteria in Cd-contaminated soils. The increase in *Azotobacter* and *Bacillus* sp. is particularly significant, as these bacteria are known for their roles in nitrogen fixation and plant growth promotion, respectively.

The observed improvements in soil microbial activity and biomass can be attributed to reduced oxidative stress and enhanced microbial resilience to heavy metals upon the application of SeNPs (Feng et al., 2021). In our study, SeNPs likely mitigated Cd toxicity, allowing microbial communities to thrive. Moreover, secreted bioactive components from PGPRs play a crucial role in enhancing soil microbial activity by improving nutrient availability and stress tolerance (Alshaal et al., 2024). The inoculation of PGPRs in our study likely enhanced microbial biomass and activity by promoting root growth and nutrient cycling, as well as by reducing the inhibitory effects of salinity and Cd on microbial communities. This is supported by the findings of Nehela et al. (2021), who demonstrated that PGPRs improved soil microbial activity and plant growth in saline soils.

The synergistic effect of SeNPs and PGPRs in our study can be explained by their complementary mechanisms. SeNPs reduce oxidative stress and heavy metal toxicity, while PGPRs enhance nutrient availability and microbial activity. This combination creates a more favorable environment for microbial growth and function, leading to improved soil health and plant resilience.

4.3. Response of soil enzyme activities to SeNPs and PGPRs under combined Cd and salinity stress

The consistent improvement in acid phosphatase activity under all treatments, particularly the combined treatment, indicates enhanced P availability in the soil. This is consistent with the findings of Alharbi et al. (2022b), who reported that SiNPs combined with PGPRs improved P availability and plant growth in saline soils. The increase in acid phosphatase activity suggests that the treatments stimulated microbial activity, leading to the mineralization of organic P into plant-available forms. This is particularly important in saline and Cd-contaminated soils, where P availability is often limited.

The increase in urease activity under the treatments reflects improved N cycling, which is crucial for plant growth. The combined treatment showed the highest urease activity, indicating a synergistic effect of SeNPs and PGPRs. This aligns with the findings of Hafez et al. (2019), who demonstrated that biochar and PGPRs enhanced urease activity and N availability in salt-affected soils. The improved urease activity likely contributed to better N mineralization and uptake by plants, which is essential for growth under stress conditions.

The enhancement of catalase activity under the treatments indicates a reduction in oxidative stress in the soil. Catalase is a key enzyme in mitigating oxidative damage caused by reactive oxygen species (ROS), which are often elevated under Cd and salinity stress. The highest catalase activity observed under the combined treatment suggests that SeNPs and PGPRs together effectively reduced oxidative stress. This is supported by the findings of Jebara et al. (2005), who reported that catalase activity increased in response to stress mitigation strategies in common bean plants under salt stress.

The modest increase in invertase activity across all treatments reflects improved C cycling potential in the soil. Invertase plays a crucial role in the breakdown of sucrose into glucose and fructose, which are essential for microbial and plant metabolism. The increase in invertase activity suggests that the treatments enhanced microbial activity and organic matter decomposition, leading to improved soil fertility. This is consistent with the findings of Kheyrodin et al. (2012), who reported that organic amendments and microbial inoculants increased invertase activity in stressed soils.

The SeNPs have been shown to reduce oxidative stress and enhance microbial activity, which in turn stimulates enzyme production (Feng et al., 2021). In our study, SeNPs likely mitigated Cd toxicity and oxidative stress, allowing microbial communities to thrive and produce more enzymes. Zhu et al. (2022) also reported that biosynthetic SeNPs alleviated heavy metal toxicity in plants and soil by improving antioxidant systems and reducing metal bioavailability. Also, PGPRs play a crucial role in enhancing soil enzyme activities by promoting microbial growth and nutrient cycling. The inoculation of PGPRs in our study likely improved enzyme activities by enhancing root exudation and organic matter decomposition, as well as by reducing the inhibitory effects of salinity and Cd on microbial communities. This is supported by the findings of Alshaal et al. (2024), who demonstrated that PGPRs improved soil enzyme activities and plant growth in saline-alkali soils.

The synergistic effect of SeNPs and PGPRs in our study can be explained by their complementary mechanisms. SeNPs reduce oxidative stress and heavy metal toxicity, while PGPRs enhance microbial activity and nutrient cycling. This combination creates a more favorable environment for enzyme production and activity, leading to improved soil health and plant resilience.

4.4. Reduction in soil Cd content and its accumulation in plant tissue

The combined treatment of SeNPs and PGPRs consistently reduced soil Cd content by approximately 34.5 % in both seasons, indicating its effectiveness in mitigating Cd contamination. This is consistent with the findings of [Shang et al. \(2024\)](#), who reported that biosynthesized SeNPs significantly reduced heavy metal concentrations in soil by enhancing metal immobilization and reducing bioavailability. Similarly, [Feng et al. \(2021\)](#) demonstrated that inorganic Se reduced Cd accumulation in soil by forming stable complexes with Cd, thereby reducing its mobility. The reduction in soil Cd content in our study can be attributed to the ability of SeNPs to immobilize Cd through the formation of insoluble Cd-Se complexes, as well as the role of PGPRs in enhancing soil microbial activity, which further stabilizes Cd in the soil.

The combined treatment also significantly reduced Cd accumulation in rice roots, shoots, and seeds, with reductions of up to 49 % in roots, 57 % in shoots, and 50 % in seeds compared to CK. This aligns with the findings of [Zhu et al. \(2022\)](#), who reported that foliar application of biosynthetic SeNPs reduced Cd, Pb, and Hg accumulation in *Brassica chinensis* by inhibiting heavy metal adsorption and improving the plant's antioxidant system. The reduction in Cd accumulation in plant tissues can be explained by several mechanisms. First, SeNPs likely reduced Cd uptake by competing with Cd for root absorption sites or by forming Cd-Se complexes that are less bioavailable. Second, PGPRs may have enhanced root exudation and microbial activity, which can immobilize Cd in the rhizosphere and reduce its translocation to shoots.

The combined treatment consistently reduced the BCF, TF, and BAC values, indicating reduced Cd mobility and bioavailability within the plant system. This is consistent with the findings of [Thongnok et al. \(2022\)](#), who reported that heavy metal-resistant PGPRs reduced Cd translocation and accumulation in rice plants by enhancing metal immobilization in the rhizosphere. The reduction in BCF, TF, and BAC values suggests that the treatments not only reduced Cd uptake by roots but also limited its translocation to shoots, thereby minimizing Cd accumulation in edible plant parts. This is particularly important for food safety, as Cd is a toxic heavy metal that poses significant health risks when ingested.

The observed reductions in soil Cd content and plant Cd accumulation can be attributed to several mechanisms. SeNPs have been shown to reduce Cd bioavailability by forming stable Cd-Se complexes, which are less mobile and less toxic to plants ([Feng et al., 2021](#)). In our study, SeNPs likely immobilized Cd in the soil, reducing its uptake by rice plants. PGPRs play a crucial role in mitigating Cd stress by enhancing root exudation, microbial activity, and metal immobilization in the rhizosphere ([Alshaal et al., 2024](#)). The inoculation of PGPRs in our study likely reduced Cd uptake and translocation by enhancing the production of metal-chelating compounds and promoting the growth of metal-resistant microbial communities.

The synergistic effect of SeNPs and PGPRs in our study can be explained by their complementary mechanisms. SeNPs reduce Cd bioavailability and oxidative stress, while PGPRs enhance metal immobilization and plant resilience. This combination creates a more effective barrier against Cd uptake and translocation, leading to reduced Cd accumulation in both soil and plant tissues.

4.5. Accumulation of selenium in plant tissues

The increase in Se content in rice tissues, particularly in seeds, highlights the role of SeNPs in enhancing the nutritional quality of rice grains. Selenium is an essential micronutrient for humans, and its deficiency is associated with various health issues, including compromised immune function and increased susceptibility to diseases ([Feng et al., 2021](#)). The application of SeNPs not only mitigated Cd toxicity but also improved the Se content in rice, making it a valuable strategy for biofortification in Cd-contaminated environments. This is consistent with the findings of [Zhu et al. \(2022\)](#), who reported that biosynthetic SeNPs improved Se content in *Brassica chinensis* while reducing heavy metal accumulation.

The combined treatment of SeNPs and PGPRs exhibited the most significant improvements in Se content, suggesting a synergistic effect. This is in line with the findings of [Shang et al. \(2024\)](#), who demonstrated that biosynthesized SeNPs combined with PGPRs enhanced Se uptake in rice while reducing heavy metal toxicity. The synergistic effect likely arises from the complementary mechanisms of SeNPs and PGPRs, where SeNPs enhance Se uptake and reduce oxidative stress, while PGPRs improve nutrient availability and plant resilience.

4.6. Osmolytes, photosynthetic pigments, antioxidant enzymes, and cell membrane stability in treated rice with SeNPs and PGPRs under combined Cd and salinity stress

The combined treatment of SeNPs and PGPRs significantly reduced Na⁺ content and increased K⁺ content in rice plants, leading to a marked improvement in the K⁺/Na⁺ ratio. This is consistent with the findings of [Alsaeedi et al. \(2018\)](#), who reported that exogenous nanosilica improved plant growth by maintaining a favorable K⁺/Na⁺ ratio under elevated Na⁺ stress. The reduction in Na⁺ content and increase in K⁺ content can be attributed to the ability of SeNPs and PGPRs to enhance ion homeostasis and reduce Na⁺ uptake by roots. This is particularly important in saline soils, where high Na⁺ levels disrupt ion balance and impair plant growth.

The significant increase in chlorophyll a, chlorophyll b, and carotenoid contents under the combined treatment indicates improved photosynthetic efficiency. This aligns with the findings of [Alharbi et al. \(2022b\)](#) who demonstrated that silica nanoparticles combined with PGPRs enhanced photosynthetic pigment contents in barley under saline conditions. The increase in photosynthetic pigments likely resulted from reduced oxidative stress and improved nutrient uptake, which are critical for maintaining photosynthetic activity under stress conditions.

The combined treatment significantly enhanced the activities of SOD, POX, CAT, and APX, indicating improved antioxidant defense mechanisms. This is consistent with the findings of [Jebara et al. \(2005\)](#), who reported that antioxidant enzyme activities increased in

common bean plants under salt stress, leading to reduced oxidative damage. The increase in SOD activity, which converts superoxide radicals into H_2O_2 , and the subsequent increase in CAT and APX activities, which detoxify H_2O_2 , suggests that the treatments effectively mitigated oxidative stress. This is supported by the findings of [Zhu et al. \(2022\)](#), who reported that biosynthetic SeNPs alleviated heavy metal toxicity by enhancing antioxidant enzyme activities in *Brassica chinensis*.

The significant reduction in MDA and H_2O_2 levels under the treatments indicates reduced oxidative stress. This is consistent with the findings of [Feng et al. \(2021\)](#), who demonstrated that inorganic selenium reduced oxidative stress by enhancing antioxidant defense mechanisms in plants exposed to heavy metals. The reduction in MDA and H_2O_2 levels can be attributed to the ability of SeNPs and PGPRs to scavenge ROS and enhance antioxidant enzyme activities.

The decrease in EL and proline content under the treatments indicates improved cell membrane stability and reduced stress. This aligns with the findings of [Nehela et al. \(2021\)](#), who reported that biochar and PGPRs enhanced membrane stability and reduced proline accumulation in maize under saline conditions. The reduction in EL and proline content suggests that the treatments effectively mitigated oxidative damage and improved plant water status, as evidenced by the increase in RWC.

The observed improvements in plant physiological and biochemical responses can be attributed to several mechanisms. First, SeNPs have been shown to reduce oxidative stress and enhance antioxidant defense mechanisms by scavenging ROS and enhancing enzyme activities ([Feng et al., 2021](#)). In our study, SeNPs likely reduced oxidative stress and improved ion homeostasis, leading to enhanced photosynthetic efficiency and membrane stability. Furthermore, PGPRs play a crucial role in mitigating stress by producing exopolysaccharides, siderophores, and phytohormones, which enhance nutrient uptake and stress tolerance ([Alshaal et al., 2024](#)). The inoculation of PGPRs in our study likely improved root growth and nutrient acquisition, as well as reduced Na^+ uptake and oxidative stress, leading to improved plant growth and resilience.

The synergistic effect of SeNPs and PGPRs in our study can be explained by their complementary mechanisms. SeNPs reduce oxidative stress and enhance antioxidant defense, while PGPRs improve nutrient uptake and stress tolerance. This combination creates a more favorable environment for plant growth and stress mitigation, leading to improved physiological and biochemical responses.

4.7. Grain production parameters of rice under Cd and salinity stress

The combined treatment of SeNPs and PGPRs consistently improved 1000-grain weight, number of grains per panicle, and grain yield across both seasons. This is consistent with the findings of [Alharbi et al. \(2022b\)](#), who reported that silica nanoparticles combined with PGPRs improved grain yield and productivity in barley under saline conditions. The increase in 1000-grain weight and number of grains per panicle suggests that the treatments enhanced nutrient uptake and photosynthetic efficiency, leading to better grain filling and higher yields. This aligns with the findings of [Hafez et al. \(2019\)](#), who demonstrated that biochar and PGPRs synergistically improved grain yield in rice under salt-affected soils by enhancing nutrient availability and stress tolerance.

The significant increase in grain yield under the combined treatment, with a 25.0 % increase over CK in 2023, underscores the effectiveness of these treatments in improving rice productivity. This is supported by the findings of [Hafez et al. \(2019\)](#), who reported that compost and PGPRs enhanced wheat yield under deficit irrigation in salt-affected soils by improving soil properties and plant physiological attributes. The increase in grain yield can be attributed to the combined effects of SeNPs and PGPRs in mitigating Cd and salinity stress, enhancing nutrient uptake, and improving photosynthetic efficiency.

The increase in leaf area under the combined treatment indicates improved photosynthetic capacity and biomass production. This is consistent with the findings of [Alsaeedi et al. \(2018\)](#), who reported that exogenous nanosilica improved plant growth by maintaining a favorable K^+/Na^+ ratio under elevated Na^+ stress. The increase in leaf area likely resulted from reduced oxidative stress and improved nutrient uptake, which are critical for maintaining photosynthetic activity and biomass production under stress conditions.

The observed improvements in grain production parameters can be attributed to several mechanisms. SeNPs have been shown to reduce oxidative stress and enhance nutrient uptake, leading to improved plant growth and yield ([Feng et al., 2021](#)). In our study, SeNPs likely mitigated Cd toxicity and oxidative stress, allowing plants to allocate more resources to grain production. This is supported by the findings of [Zhu et al. \(2022\)](#), who reported that biosynthetic SeNPs alleviated heavy metal toxicity in *Brassica chinensis* by improving antioxidant systems and reducing metal uptake. PGPRs play a crucial role in enhancing nutrient uptake and stress tolerance by producing exopolysaccharides, siderophores, and phytohormones ([Alshaal et al., 2024](#)). The inoculation of PGPRs in our study likely improved root growth and nutrient acquisition, as well as reduced Na^+ uptake and oxidative stress, leading to improved grain yield and productivity. This is consistent with the findings of [Nehela et al. \(2021\)](#), who demonstrated that biochar and PGPRs enhanced maize resilience to water salinity by improving nutrient uptake and stress tolerance.

The synergistic effect of SeNPs and PGPRs in our study can be explained by their complementary mechanisms. SeNPs reduce oxidative stress and enhance nutrient uptake, while PGPRs improve root growth and stress tolerance. This combination creates a more favorable environment for plant growth and grain production, leading to higher yields and improved productivity.

5. Conclusion

This study demonstrates the significant potential of combining PGPRs and SeNPs as a sustainable and innovative approach to remediate saline Cd-contaminated soils and enhance rice productivity. The synergistic effects of microbial consortia and nanoparticles not only improved soil health by reducing salinity and Cd bioavailability but also enhanced rice physiological traits, antioxidant enzyme activities, and grain yield. The combined treatment resulted in a 25 % increase in grain yield, highlighting its effectiveness in mitigating the adverse effects of salinity and heavy metal stress. Furthermore, the reduction in Cd accumulation in rice seeds and the increase in Se content in shoots and seeds underscore the dual benefits of this approach in ensuring food safety and enhancing the

nutritional quality of rice. However, the study has limitations, including the need for long-term field trials to assess the persistence of these effects and the potential environmental impacts of nanoparticle applications. Future research should explore the mechanisms underlying microbial-nanoparticle interactions, optimize application methods, and evaluate the scalability of this approach in diverse agroecological zones. This research paves the way for sustainable soil management practices that can address the growing challenges of soil degradation and food security in contaminated environments.

CRedit authorship contribution statement

Gao Yan: Investigation. **Hafez Emad M.:** Conceptualization. **Alshaal Tarek:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization. **Omara Alaa El-Dein:** Methodology. **Hamada Maha M.:** Data curation. **Alharbi Khadiga:** Project administration. **La Honggui:** Formal analysis.

Author agreement

All authors have seen and approved the final version of the manuscript being submitted. Authors warrant that the article is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Khadiga Alharbi reports a relationship with Princess Nourah bint Abdulrahman University that includes: board membership. If there are other authors, they 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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Data availability

Data will be made available on request.

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