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Strengthen sunflowers resilience to cadmium in saline-alkali soil by PGPR-augmented biochar

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ABSTRACT

In the center of the Nile Delta in Egypt, the Kitchener drain as the primary drainage discharges about 1.9 billion m^3 per year of water, which comprises agricultural drainage (75 %), domestic water (23 %), and industrial water (2 %), to the Mediterranean Sea. Cadmium (Cd) stands out as a significant contaminant in this drain; therefore, this study aimed to assess the integration of biochar (0, 5, and 10 ton ha^{-1}) and three PGPRs (PGPR-1, PGPR-2, and PGPR-3) to alleviate the negative impacts of Cd on sunflowers (*Helianthus annuus* L.) in saline-alkali soil. The treatment of biochar (10 ton ha^{-1}) and PGPR-3 enhanced the soil respiration, dehydrogenase, nitrogenase, and phosphatase activities by 137 %, 129 %, 326 %, and 127 %, while it declined soil electrical conductivity and available Cd content by 31.7 % and 61.3 %. Also, it decreased Cd content in root, shoot, and seed by 55.3 %, 50.7 %, and 92.5 %, and biological concentration and translocation factors by 55 % and 5 %. It also declined the proline, lipid peroxidation, H_2O_2, and electrolyte leakage contents by 48 %, 94 %, 80 %, and 76 %, whereas increased the catalase, peroxidase, superoxide dismutase, and polyphenol oxidase activities by 80 %, 79 %, 61 %, and 116 %. Same treatment increased seed and oil yields increased by 76.1 % and 76.2 %. The unique aspect of this research is its investigation into the utilization of biochar in saline-alkali soil conditions, coupled with the combined application of biochar and PGPR to mitigate the adverse effects of Cd contamination on sunflower cultivation in saline-alkali soil.

1. Introduction

Sunflower (*Helianthus annuus* L.) is cultivated on a large scale for their oil-rich seeds. The global demand for sunflower oil contributes significantly to the agricultural economy. Sunflower seeds are a popular snack and ingredient in the food industry. They are used in various products such as bakery goods, confectionery items, salads, and snack bars, which add value to the food manufacturing sector (Adeleke and Babalola, 2020).

Kitchener drain – also known as El-Gharbiya main drain – is likely one of the primary drains responsible for collecting drainage water in the center of the Nile Delta, Egypt, and conveying it ultimately to the Mediterranean Sea. It might be part of a broader network of drainage infrastructure designed to control water flow and protect agricultural lands from water-related issues (El-Amier et al., 2018). It covers a watershed of around 472,500 acres (El Gammal, 2016). It releases approximately 1.9 billion m^3 of drainage water annually, which comprises mainly agricultural drainage (75 %), domestic water (23 %), and industrial water (2 %), mostly from textile factories in El-Mahalla El-Kubra city, El-Gharbeyia, Egypt (Abdel Rashid, 2002). As a result, the drainage water gets polluted with salts, agro-chemicals (e.g., pesticides, herbicides, and trace elements), and diverse pathogens arising from sewage water (Gad and Fadl, 2015). Due to the lack of freshwater supplies, farmers resort to using Kitchener's drainage water to irrigate

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their agricultural fields, which are approximately accounted for 195, 000 ha. However, if suitable treatment plans are not implemented, reusing drainage water could have serious adverse effects on soil, crops, animals, and human health (Zahran et al., 2015).

Cadmium (Cd) is a prevalent inorganic pollutant stemming from diverse industrial activities, mining operations, and agricultural practices (Kabata-Pendias, 2011). A noteworthy origin of Cd in the agricultural environment arises from the utilization of water contaminated with Cd, especially drainage water. In regions with poorly managed drainage systems or where industrial discharges mix with water sources, the risk of Cd accumulation in irrigation water becomes a critical concern (El-Hameed et al., 2021). The necessity of Cd for plant growth has not been conclusively demonstrated; however, plants can uptake large quantities of Cd through their root and/ or foliar systems. A large fraction of Cd is passively absorbed by root systems; nevertheless, Cd could be also actively absorbed (Smeyers-Verbeke et al., 1978). Various soil factors regulate the absorption and buildup of Cd in plant organs, including soil pH, redox potential, Cl^- concentration in soil solution, soil texture, soil salinity, rhizosphere microbes, and root exudates (Abedi and Mojiri, 2020). Electrical conductivity (EC_e) of soil solution, as an indicator of soil salinity, probably lead soil pH impacts on the uptake of Cd. For example, sunflower and potato plants were reported to absorb higher quantities of Cd from saline soils (Nriagu, 1998). This might be ascribed to the increased mobility and phytoavailability of Cd after formation of strong complexes with Cl^- (Mani et al., 2007).

Typical indications of Cd toxicity in plants encompass chlorosis, inhibited growth, necrosis, leaf curling, diminished flowering and fruit yield, imbalances in nutrients, restricted root development, wilting, modified photosynthesis, heightened production of reactive oxygen species (ROS), and damage to cell membranes (Li et al., 2023). The phytotoxic threshold of Cd in plants spans from 5 to 20 mg kg^{-1} (Macnicol and Beckett, 1985). The simultaneous presence of Cd contamination in irrigation water and soil affected by salinity presents a substantial challenge for sustainable agriculture, as it has the potential to significantly jeopardize crop productivity and compromise food safety. Applying biochar, a carbon-rich material, and plant growth-promoting rhizobacteria (PGPR) presents a potent and sustainable method for remediating soils polluted with Cd. This innovative strategy addresses the complex challenges posed by Cd pollution and salinity, providing a range of benefits that improve soil health, enhance plant growth, and promote long-term environmental sustainability (De Andrade et al., 2023; Meng et al., 2022).

Biochar is defined by its elevated carbon content, a result of the pyrolysis of plant residues under oxygen-restricted conditions within the temperature range of 300–500 °C (Armah et al., 2023). Biochar plays a crucial role in mitigating the harmful impacts of heavy metal contamination on the growth of crops, especially those cultivated in soil affected by salinity. This sustainable soil amendment offers numerous benefits that contribute to improved plant resilience and growth under challenging environmental conditions (Gogoi et al., 2021). Employing biochar as a soil amendment in salt-affected soil offers a comprehensive strategy to mitigate the adverse impacts of Cd contamination on plant growth, primarily through key factors like Cd immobilization (Meng et al., 2022), enhanced nutrient retention (Zhang et al., 2021), improved soil structure and aeration (Singh Yadav et al., 2023), regulation of soil pH (Geng et al., 2022), alleviation of salinity stress (Karabay et al., 2021), induction of antioxidant defense (Haider et al., 2022), and promotion of microbial activity (Wang et al., 2022).

PGPR provide a variety of services that improve plant health, nutrient absorption, and stress resilience, especially in the face of abiotic stress (Khatoon et al., 2022). The importance of PGPR in this context can be understood through several key mechanisms, such as Cd detoxification (El-Nahrawy et al., 2019; Halim et al., 2020), enhanced nutrient availability (De Andrade et al., 2023), induction of phytohormones, relieving oxidative damage (Desoky et al., 2020), improvement of salt resistance (Egamberdieva et al., 2019), control of diseases (Wang et al., 2021), enhanced root growth and mycorrhizal associations (Germida and Walley, 1996), and phytostimulation (Upadhyay et al., 2022). PGPR strains, such as *Azospirrillum, Bacillus* and *Pseudomonas*, are well-known to withstand and remediate environmental pollutants alongside enhancing plant growth (Cruz-Hernández et al., 2022; El-Nahrawy et al., 2019). PGPR application could modulate soil physicochemical properties and convert heavy metal contaminations to a less toxic state by reduction, oxidation, methylation or de-methylation (Wang et al., 2022).

The utilization of biochar in less weathered temperate and arid environments, notable for their alkaline soil pH, represents a relatively recent development. Consequently, the novelty of this study lies in its exploration of biochar application in alkaline soil conditions, alongside the combined use of biochar and plant growth-promoting rhizobacteria (PGPR). This investigation aims to alleviate the detrimental impacts of cadmium (Cd) contamination on sunflowers (*Helianthus annuus* L.) cultivated in sodic-saline soil. The research also examined the impact of Cd in irrigation water on various aspects of sunflower growth, physiological parameters, and the absorption and accumulation of Cd in different plant tissues. Moreover, the study explored the potential of sunflowers for phytoremediation by evaluating the Cd sequestration capacity of the plant in a controlled experimental setting.

2. Materials and methods

2.1. Experimental site

Open field trials were carried out at the Taawun Village 1, El-Hamool county (31.40° N, 31.17° E), Kafr El-Sheikh governorate, Egypt, in 2022. The climate in this area is marked by a Subtropical desert climate, and the corresponding climate data is as follows (on average): high air temperature, 35.07 °C (in August); low air temperature, 8.78 °C (in January); annual precipitation, 4.39 mm (mainly in winter season); number of days with rainfall ($\geq$ 1.0 mm), 13.29 days; humidity, 62.16 %; and wind speed, 4.18 km h^{-1}. The main source of water used in irrigation of agricultural crops is the water of Kitchener drain, with the following properties in 2022 as described by the Soil Improvement and Conservation Department, Agricultural Research Center, Giza, Egypt: pH, 7.28±0.01; EC, 0.55±0.02 dS m^{-1}; SAR (sodium adsorption ratio), 1.46 ±0.04; Na^+, 1.98±0.06 mg L^{-1}; Cl^-, 3.45±0.07 mg L^{-1}; SO_4^{2-}, 0.12 ±0.01 mg L^{-1}; NH_4^+, 1.71±0.03 mg L^{-1}; Cd, 0.094±0.02 mg L^{-1}; Pb, 0.591±0.06 mg L^{-1}; and Ni, 0.099±0.07 mg L^{-1}. The recommended maximum level of Cd in irrigation water set by FAO (Food and Agriculture Organization) is 0.01 mg L^{-1} (Ayers and Westcot, 1985); therefore, the used water in the present study is classified as Cd-polluted irrigation water since Cd concentration is about 9-fold higher than the recommended level. In this study, the soil utilized is categorized as clayey soil, and its physicochemical attributes are delineated by the Soil Improvement and Conservation Department at the Agricultural Research Center in Giza, Egypt, as follows: pH (1:2.5 soil: water suspension), 8.22±0.02; EC_e (soil paste extract), 4.61±0.01 dS m^{-1}; SOM (soil organic matter), 11.2±0.03 g kg^{-1}; ESP (exchangeable sodium percentage), 22.6±0.42 %; Ca^{2+}, 7.54±0.94 meq L^{-1}; Mg^{2+}, 5.76 ±1.11 meq L^{-1}; Na^+, 26.75±2.06 meq L^{-1}; K^+, 0.33±0.02 meq L^{-1}; HCO_3^-, 4.61±0.56 meq L^{-1}; Cl^-, 24.56±1.11 meq L^{-1}; SO_4^{2-}, 15.13 ±3.03 meq L^{-1}; available N, 9.70±0.91 mg kg^{-1}; available P, 8.24 ±1.33 mg kg^{-1}; available K, 344±26.42 mg kg^{-1}; total Cd, 5.16 ±0.18 mg kg^{-1}; and available Cd, 0.96±0.04 mg kg^{-1}. Soil samples were collected using a stainless-steel soil auger at a depth of 0–30 cm prior to the commencement of the experiment. The Cd content in soil is influenced by its texture, leading to variations in the maximum permissible soil Cd content based on the specific soil texture. In light-textured soils, the critical Cd content is 5.33 mg kg^{-1}, in medium-textured soils, it is 6.33 mg kg^{-1}, and heavy-textured soils exhibit a higher critical Cd content of 9.29 mg kg^{-1}. Hence, soils characterized by elevated clay content exhibit a higher critical cadmium (Cd) content (Sukarjo et al., 2019). The experimental soil, being clayey with a total Cd content of 5.16±0.18 mg kg^{-1}, is presently not classified as Cd-polluted soil. Nevertheless, prolonged use of irrigation water contaminated with Cd could eventually lead to its classification as Cd-polluted soil.

2.2. Experimental format

The experiments were organized using a Completely Randomized Factorial Design, and each experimental condition was replicated four times. Biochar was applied at three levels, i.e., zero, 5, and 10 ton ha^{-1}, in the main plots, while four PGPR treatments, i.e., CK (uninoculated plot), PGPR-1 (*Azospirillum brasilense* SARS 1001 + *Bacillus circulans* NCAIM B.02324), PGPR-2 (*Azospirillum brasilense* SARS 1001 + *Pseudomonas koreensis* MG209738), and PGPR-3 (*Azospirillum brasilense* SARS 1001 + *Bacillus circulans* NCAIM B.02324 + *Pseudomonas koreensis* MG209738), were occupied the sub-plots, with a total of 12 combinations. The experimental plot had dimensions of 10.5 m^2 (3×3.5 m), comprising six rows spaced 50 cm apart.

2.2.1. Sources and preparation of PGPR

PGPR strains, specifically *Azospirillum brasilense* SARS 1001, *Bacillus circulans* NCAIM B.02324, and *Pseudomonas koreensis* MG209738, were sourced from the Department of Agricultural Microbiology at the Soils, Water, and Environment Research Institute (SWERI), ARC, Egypt. These strains were chosen for their unique plant growth-promoting characteristics and demonstrated tolerance to Cd, as evidenced by a minimum inhibitory concentration of 500 mg L^{-1}. The bacterial growth was facilitated using semi-solid malate medium specifically for *A. brasilense* SARS 1001 (Döbereiner and Day, 1976)**,** the nutrient broth medium for the *B. circulans* NCAIM B.02324 (Atlas and Atlas, 2004)**,** and the King's B (KB) broth medium for *P. koreensis* MG209738 (Johnsen and Nielsen, 1999). Before preparing the inoculum, the chosen PGPR strains were cultivated in dedicated liquid growth media and placed on a rotary shaker at 150 rpm for 27 h. Inocula based on peat were created by combining 15 mL of 10^8 CFU mL^{-1} from each culture with 30 g of the sterilized carrier. Sunflower seeds were sterilized and subsequently inoculated with PGPR inocula at a rate of 950 g ha^{-1}, away from direct sunlight, just before sowing.

2.2.2. Source and characteristics of biochar

The biochar employed in this study was produced through the pyrolysis of two feedstocks, namely rice husk and corn stalk, in a 1:1 ratio (on a dry mass basis) at 450 °C for 3 h in a muffle furnace under oxygen-free conditions. The physicochemical features of prepared biochar were as follows: pH (1:5, biochar: water suspension), 7.60±0.02; EC (1:5, biochar: water extract), 0.70±0.01 dS m^{-1}; $CaCO_3$, 1.4±0.03 %; bulk density, 0.20±0.03 g cm^{-3}; specific surface area, 37.0±2.13 m^2 g^{-1}, water holding capacity, 35.01±2.23 %; moisture content, 11.4±1.09 %; N, 25.21±2.91 mg kg^{-1}; P, 7.45±0.83 mg kg^{-1}; K, 13.21±1.42 mg kg^{-1}; and Cd, 0.06±0.001 mg kg^{-1}.

2.2.3. Growth conditions

Sunflower (*Helianthus annuus* L., cv. Sakha 53) seeds were sourced from the Oil Crop Research Center, Agricultural Research Center in Giza, Egypt. The seeds were planted at a rate of 12 kg ha^{-1} on May 19 and harvested on September 5, 2022. The sowing process involved placing 3–5 seeds per hill, with a plant spacing of 30 cm on one ridge, resulting in a plant density of 57,000 plants ha^{-1}. After fifteen days from the initial seed sowing (DAS), the seedlings were selectively thinned to maintain one plant per hill. Kitchener drain water was used for irrigation. Throughout the growth season, plants received a total of six surface irrigations at 15-day intervals, following the recommendations of the Ministry of Agriculture and Land Reclamation in Egypt. Prior to sowing, the soil underwent two plowing sessions, incorporating calcium superphosphate fertilizer at a rate of 36 kg ha^{-1} during the second tillage. Nitrogen fertilization, in the form of ammonium nitrate (33.5 % N), was administered at a rate of 80 kg N ha^{-1}, split into two equal doses during the first and second irrigations. Additionally, potassium fertilization at a rate of 58 kg ha^{-1} (K_2O) was applied concurrently with the second dose of nitrogen fertilizer. Weed control was managed using Stomp herbicide at a rate of 3 L ha^{-1}. Furthermore, manual removal of emerging weeds was performed 30 days after seed sowing.

2.3. Soil-related parameters

2.3.1. Soil sampling and determination of pH, EC, and available Cd

Soil samples for determining chemical properties such as pH, EC, and available Cd content were gathered post-plant harvest (109 DAS). Three soil cores (5 cm diameter, 0–20 cm depth) were taken from each experimental unit and thoroughly mixed to create a composite soil sample per plot. After eliminating stones, gravel, and visible plant residues, the soil samples were air-dried, homogenized, sifted through a 2 mm sieve, and stored in polyethylene bags at room temperature for chemical analyses. In contrast, soil samples intended for biochemical analysis, including soil respiration and soil enzyme activities, were obtained at 80 DAS in sterile polyethylene containers. These samples were promptly transferred to the laboratory in an ice storage box. Three subsamples were collected from each plot (5 cm diameter, 0–20 cm) and combined to create a representative soil sample per plot. In the laboratory, the soil samples were examined for the presence of plant detritus, large soil aggregates, and stones. Subsequently, they were sieved using a 5 mm sieve and stored in polyethylene bags in a −20 ºC freezer for further analyses. Soil:distilled water suspension (1:2.5 w/w) was applied for determination of soil pH using pH-meter (Jenway 3510, USA), while soil EC_e was measured in soil past extract at 23±2 ºC by EC-meter (Jenway 4310, USA) (Sparks, 1996). EDTA was employed to extract available soil Cd content (mg kg^{-1}), which was quantified using Atomic Absorption Spectrophotometry (AAS, PERKIN ELMER 3300, USA) with a detection limit of 10 ppb (Jackson, 1973).

2.3.2. Determination of soil respiration and enzyme activities

After a 10-day incubation period at 35 ºC with NaOH-trapping glucose-induced soil respiration (mg CO_2 100 g^{-1} dry soil 24 h^{-1}) was determined (Ölinger et al., 1996). Before measuring soil respiration, frozen soil samples were thawed for 24 h and then amended with glucose at a rate of 80 mg g^{-1}. The soil's moisture content was regulated to reach 60 % of its total water capacity, aiming to encourage microbial growth after the dormancy period.

The soil's dehydrogenase activity (μg TPF g^{-1} dry soil 24 h^{-1}) was assessed through the reduction of 2,3,5-triphenyl tetrazolium chloride (3 % w/v TTC) method (Chander and Brookes, 1991). The estimation of soil phosphatase activity (mg phenol g^{-1} dry soil 2 hr^{-1}) followed the procedure outlined by (Dick et al., 2000).

2.4. Plant-related parameters

2.4.1. Sampling and sample preparation of plant tissues

At the harvest stage (109 DAS), plant samples were collected randomly at a rate of five plants per plot, totaling up to 20 plants per treatment. These plant samples were divided into roots, shoots, and seeds. After washing the plant tissues with distilled water to eliminate any surface contaminants, the samples were air-dried, subjected to a hot-air oven at 70 ºC for 24 h, pulverized into a fine powder using a bead mill (model: EDW-50, Shanghai, China), and stored in polyethylene bags for subsequent analyses. Root samples underwent a wash with 1 M HCl, followed by thorough rinsing with distilled water (Humphries, 1956).

2.4.2. Analysis of Cd content in plant samples

Plant samples underwent wet digestion in a 250-mL Kjeldahl digestion tube by combining 500 mg of fine plant tissue powder with 9 mL of concentrated HNO_3. The digestion tube was then transferred to a Tecator Digestion unit (VELP Digester model DK 42/26, VELP scientific

Ltd., Italy), and the samples were heated to 120 °C for 60 min. Following cooling (below 100 °C), 3 mL of concentrated $HClO_4$ was added, and the samples were heated to 150 °C for an additional 60 min. The total volume of the digested mixture was adjusted to 25 mL using distilled water, and the solution was filtered using Whatman filter paper 40. The concentration of Cd was determined in the clear filtrate using Atomic Absorption Spectrophotometry (AAS, Perkin Elmer 3300, USA) with a detection limit of 10 ppb (Ryan et al., 2001).

2.4.3. Computation of BCF, TF, and BAC indices

Biological concentration factor (BCF), translocation factor (TF), and biological accumulation coefficient (BAC) were determined through the following calculations:

$BCF = \frac{Cd\ content\ in\ plant\ root}{Cd\ content\ in\ soil}$ (Yoon et al., 2006)

$TF = \frac{Cd\ content\ in\ plant\ shoot}{Cd\ content\ in\ plant\ root}$ (Cui et al., 2007)

$BAC = \frac{Cd\ content\ in\ plant\ shoot}{Cd\ content\ in\ soil}$ (Li et al., 2007)

2.4.4. Determination of stomatal conductance

RWC, EL, proline, MDA, and H_2O_2The plant specimens employed to assess stomatal conductance, relative water content (RWC), electrolyte leakage (EL), proline, malondialdehyde (MDA), and hydrogen peroxide (H2O2) were obtained from the fully expanded leaves located at the plant tip. Sampling was conducted 80 days after the seeds were sown. Stomatal conductance (mmol H_2O m^{-2} s^{-1}) was determined during the period from 12:00–14:00 h, consistently utilizing the youngest fully expanded top leaf exposed to high light intensity (PAR $\geq$ 1200μmol $m^{-2}s^{-1}$). The measurements were conducted using LI-6400 portable gas exchange systems (Li-COR, Lincoln, NE, USA).

For RWC (%) determination, 1 cm^2 leaf discs were employed, and their fresh weight (FW) was promptly measured. Following a 5-h soaking period in distilled water, the turgid weight (TW) was recorded. The dry weight (DW) was determined by subjecting the leaf discs to oven-drying at 70 °C for 48 h. The RWC was calculated using the following formula:$RWC(\%) = \frac{FW-DW}{TW-DW}x100$ (Barrs and Weatherley, 1962)

Ten leaf discs measuring 1 cm^2 each were gathered and rinsed with distilled water for the evaluation of electrolyte leakage (EL %). Subsequently, these samples were placed in test tubes filled with 10 mL of distilled water. The EL percentage was computed using the following formula:

$EL(\%) = \frac{C1}{C2} \times 100$ (Bajji et al., 2002)

where, C1 denotes the electrical conductivity (EC) of samples subjected to a water bath at 55 °C for 25 min, while C2 signifies the EC of the identical samples exposed to a water bath at 100 °C for 10 min. The leaf proline content (mg 100 g^{-1} FW) was determined using the method developed by (Bates et al., 1973). In summary, 0.5 g of plant leaves was ground with 3 % sulfuric acid and subsequently centrifuged at 12,000 g for 5 minutes. The resulting solution was quantified using ninhydrin reagent. The obtained supernatant was then combined with toluene, and the absorbance was measured at 520 nm using the Shimadzu UV-160A spectrophotometer (Japan).

MDA (nmol g^{-1} FW) is the end product of lipid peroxidation, and its content was determined through the thiobarbituric acid test (TBA) following the method outlined by (Du and Bramlage, 1992). In summary, 500 mg of plant leaves was homogenized and ground in liquid nitrogen with hydro-acetone buffer (4:1 v/v). Subsequently, a solution of 20 % trichloroacetic acid (TCA) and 0.01 % butyl hydroxyl toluene (BHT) was added, and the samples were incubated at 95 °C. After incubation, the homogenized samples underwent centrifugation at 10, 000 g for 10 min. The absorbance was then measured spectrophotometrically at 532 nm and 600 nm using the Shimadzu UV-160A spectrophotometer (Japan).

The hydrogen peroxide (H_2O_2; μmol g^{-1} FW) content in 1 g of plant leaves was determined according to the method described by (Velikova et al., 2000). The extraction process involved the use of liquid nitrogen and trichloroacetic acid (TCA: 0.1 %), followed by centrifugation at 6000 g for 15 min. The concentration of the yellow color in the supernatant was measured at 426 nm using the Shimadzu UV-160A spectrophotometer (Japan) for the colorimetric analysis of H_2O_2.

2.4.5. Pigments of photosynthetic molecules and photosynthetic rate

Photosynthetic pigments (mg g^{-1} FW) were evaluated 80 DAS, utilizing the topmost fully expanded leaves, following the methodology outlined by (Lichtenthaler, 1987). The concentrations of chlorophyll a (chl a), chlorophyll *b* (chl b), and carotenoids in the solution were calculated using the following equations:

$\text{chl a} = 12.7\ (A_{663}) - 2.69\ (A_{645})$

$\text{chl b} = 25.8\ (A_{645}) - 4.68\ (A_{663})$

$\text{carotenoids} = (1000\ (A_{470}) - 2.27\ (\text{chl a}) - 81.4\ (\text{chl b}))\ /\ 227$

At 80 days post-sowing, the photosynthetic rate was assessed in the uppermost fully-expanded leaves collected from the plant tip. The measurement was carried out under optimal conditions, with an irradiance of 1000 μmol m^{-2} s^{-1}, utilizing a LI-6400 portable photosynthesis device (Li-COR, Lincoln, NE, USA) at a temperature of $30\pm2°C$. The experiment maintained a CO_2 concentration ranging from 350 to 400 μmol mol^{-1}, along with a vapor pressure deficit (VPD) corresponding to 50 % relative humidity (RH) (Tian-gen et al., 2017).

2.4.6. Determination of antioxidant enzyme activities

Leaf samples (1 g), collected at 80 DAS, were homogenized in a cooled Tris-HCl buffer (0.1 mol L^{-1}, pH 7.8), containing 1 mmol L^{-1} EDTA, 1 mmol L^{-1} dithiothreitol, and 5 mL polyvinyl pyrrolidone (4 %) for the assessment of antioxidant enzyme activity, with three replicates used for each measurement. The activity of catalase (CAT; μmol H_2O_2 g^{-1} FW min^{-1}) was determined following the method described by (Aebi, 1984). Superoxide dismutases (SOD) activity (SOD; μmol H_2O_2 min^{-1} g^{-1} FW) was measured following the method outlined by (Beauchamp and Fridovich, 1971). Peroxidase (POD; μM tetraguaiacol g^{-1} FW min^{-1}) activity was measured as described by (Vetter et al., 1958). Polyphenol oxidase (PPO; μM tetraguaiacol g^{-1} FW min^{-1}) activity was assessed according to the procedure outlined by (Taranto et al., 2017).

2.4.7. Oil yield and quality

Upon reaching maturity, indicated by the yellowing of the back of the head and browning of the bracts (109 DAS), five chosen plants were harvested from each plot. The harvested heads underwent complete drying during storage, and the seeds were manually threshed and allowed to dry. The weight of 100 seeds (g) and the seed yield adjusted to kg ha^{-1} were measured with a moisture basis of 10 %. To determine the oil percentage (% dry matter) in the seed samples, a Soxhlet extractor was employed following the method outlined by (AOAC, 2016). The oil yield (kg ha^{-1}) was subsequently calculated by multiplying the seed yield (kg ha^{-1}) by the seed oil content.

2.5. Statistical analysis

The analysis of data was performed using Microsoft Excel 2016 and the SPSS 25.0 software package (SPSS Inc., Chicago, IL, USA). Separate one-way ANOVA tests were conducted for both the biochar and PRGPs treatments. Post-hoc tests, specifically Tukey's test, were utilized to compare means, and significant differences were considered at the $p \leq 0.05$ level. The data were presented in the format of mean ± standard deviation.

3. Results

3.1. Alterations in soil chemical and biochemical activities

Experimental plots, including CK, displayed lower pH values than soil before starting the experiment (Table 1). This reduction in soil pH was slight; however, the treatment of 10 ton ha^{-1} biochar+PGPR-3 exhibited the lowest pH. The CK plots revealed higher EC$_e$ values than the initial EC$_e$ value before the onset of the experiment. Yet, all treatments of biochar and PGPRs reduced EC$_e$. The integration between biochar (10 ton ha^{-1}) and PGRP-3 possessed the lowest EC$_e$ of 3.34 dS m^{-1}. Irrigation sunflowers with Cd-polluted water without precautions increased the available soil Cd content (1.06 mg kg^{-1}) above basic value before starting the experiment (0.96 mg kg^{-1}). Both biochar and PGPRs application significantly reduced the available soil Cd content achieving the highest reduction when applied at the rate of 10 ton ha^{-1}+PGPR-3.

Biochar and PGPR application increased soil respiration (mg CO$_2$ 100 g^{-1} soil 24 h^{-1}) compared to CK. For instance, treated plots with 5 and 10 ton ha^{-1} of biochar had soil respiration of 35.5 and 43.0, respectively, while CK plots recorded 26.7. PGPR-1 and PGPR-2 showed almost the same soil respiration rate, whereas PGPR-3 displayed the highest soil respiration rate. The most noticeable improvement in soil respiration rate corresponded to the treatment of 10 ton ha^{-1} biochar+PGPR-3, recording the highest soil respiration rate of 48.6.

Biochar-amended plots displayed high soil dehydrogenase activities (μg TPF g^{-1} dry soil 24 h^{-1}), where 10 ton ha^{-1} biochar showed soil dehydrogenase activity of 37.5. The PGPRs also induced the soil dehydrogenase activity, recording higher activities than the CK; for instance, PGPR-2 displayed an activity of 33.4. However, the integration between 10 ton biochar ha^{-1} and PGPR-3 revealed the highest soil dehydrogenase (41.1).

The soil nitrogenase activity (μM C$_2$H$_4$ kg^{-1} soil h^{-1}) gradually increased upon the increase in the rate of applied biochar. While soil nitrogenase activity in biochar-amended plots with 10 ton ha^{-1} was 178, CK plots displayed a nitrogenase activity of 76. Inoculated plots with PGPRs revealed higher soil nitrogenase activities than the CK plots. Results also proved that the dual application of PGPRs and biochar at the rate of 10 ton ha^{-1} was the best, recording the highest activities of soil nitrogenase. CK plots had a soil nitrogenase activity of 76, while biochar-amended plots with 10 ton ha^{-1} and inoculated with PGPR-3 displayed an activity of 196.

The application of biochar at the rate of 10 tons ha^{-1} resulted in the highest soil phosphatase activity (0.85), whereas CK plots exhibited the lowest activity (0.57). Similarly, inoculation with PGPRs led to an

Table 1
Alterations in soil chemical and biochemical activities after irrigation of sunflower (*Helianthus annuus* L., cv. Sakha 53) with cadmium-polluted drainage water in the presence of biochar (0, 5, and 10 ton ha^{-1}) and different PGPRs inocula, i.e., PGPR-1 (*Azospirillum brasilense* SARS 1001+*Bacillus circulans* NCAIM B.02324), PGPR-2 (*Azospirillum brasilense* SARS 1001+*Pseudomonas koreensis* MG209738), and PGPR-3 (*Azospirillum brasilense* SARS 1001+*Bacillus circulans* NCAIM B.02324+*Pseudomonas koreensis* MG209738).

		pH†	EC$_e^{‡}$	Cd	Soil respiration	Dehydrogenase	Nitrogenase	Phosphatase
			dS m^{-1}	mg kg^{-1}	(mg CO$_2$ 100 g^{-1} soil 24 h^{-1})	(μg TPF g^{-1} dry soil 24 h^{-1})	(μM C$_2$H$_4$ kg^{-1} soil h^{-1})	(mg phenol g^{-1} soil 24 h^{-1})
Biochar	0	8.14±0.01 ab	4.23±0.03 a	0.71±0.00 a	26.7±4.06c	22.0±2.76c	76±19.22c	0.57±0.10c
	5	8.12±0.02 b	4.01±0.04 b	0.50±0.01 b	35.5±5.48 b	29.4±3.80 b	128±27.11 b	0.72±0.06 b
	10	8.12±0.01 b	3.88±0.04c	0.43±0.01c	43.0±4.50 a	37.5±3.45 a	178±20.05 a	0.85±0.06 a
PGPR	CK$^{\Phi}$	8.16±0.01 ab	4.63±0.04 a	0.69±0.02 a	28.1±7.11 b	24.7±6.22 b	94±43.65 b	0.60±0.16 b
	PGPR-1	8.13±0.01 b	4.21±0.04 b	0.53±0.00 b	35.5±6.74 ab	29.5±6.93 ab	128±44.75 ab	0.72±0.11 ab
	PGPR-2	8.12±0.01 bc	3.84±0.04c	0.50±0.00 b	36.4±7.03 ab	31.0±6.98 ab	134±45.68 ab	0.73±0.10 ab
	PGPR-3	8.09±0.02c	3.48±0.03 d	0.46±0.01c	40.3±7.72 a	33.4±6.94 a	152±44.46 a	0.80±0.12 a
Interaction								
0-Biochar	CK	8.18±0.01 a	4.89±0.05 a	1.06±0.01 a	20.5±0.93 g	17.9±0.46 k	46±1.44 i	0.41±0.02 h
	PGPR-1	8.14±0.01 abc	4.41±0.03c	0.64±0.00 b	27.4±0.30 f	21.9±0.51 j	78±2.15 h	0.60±0.01 g
	PGPR-2	8.13±0.01 bcd	3.93±0.03 ef	0.61±0.00 b	27.9±0.92 f	23.0±0.46 ij	83±1.48 h	0.62±0.01 fg
	PGPR-3	8.11±0.01 cde	3.69 ±0.04 h	0.53 ±0.00 cd	30.9±0.66 e	25.1±0.37 h	96±0.76 g	0.65±0.01 f
5-Biochar	CK	8.17±0.01 ab	4.60±0.03 b	0.56±0.04c	27.0±0.58 f	24.0±0.19 hi	91±2.20 g	0.63±0.01 f
	PGPR-1	8.13±0.01 abcd	4.18±0.06 d	0.51±0.00 de	36.4±0.62 d	28.8±0.51 g	124±2.78 f	0.71±0.01 e
	PGPR-2	8.11±0.01 cde	3.83±0.05 fg	0.48±0.00 ef	37.1±0.12 d	30.8±0.65 f	132±3.05 e	0.72±0.01 e
	PGPR-3	8.09±0.06 de	3.42±0.04 i	0.45±0.00 fgh	41.3±0.67c	34.0±0.34 d	164±3.77c	0.81±0.02c
10-Biochar	CK	8.15 ±0.01abc	4.40±0.03c	0.46±0.01 fg	36.7±1.00 d	32.2±0.15 e	146±3.83 d	0.76±0.01 d
	PGPR-1	8.12±0.01 bcd	4.05±0.03 e	0.43±0.01 ghi	42.8±0.79 bc	37.9±0.21 c	181±4.82 b	0.84±0.01 b
	PGPR-2	8.11 ±0.01cde	3.74±0.05 gh	0.42±0.01 hi	44.1±0.68 b	39.1±0.17 b	188±1.34 ab	0.85±0.01 b
	PGPR-3	8.06±0.01 e	3.34±0.05 I	0.41±0.01 i	48.6±0.62 a	41.1±0.27 a	196±3.14 a	0.93±0.01 a

Means in the same column followed by different letters are significant according to the Tukey's test ($P \leq 0.05$). Data are Means ± SD and n = 3. $^{\Phi}$ control, † soil pH (1:2.5 soil:distilled water suspension), ‡ electrical conductivity (soil paste extract) increase in soil phosphatase activity compared to CK. Although the differences among PGPRs were not statistically significant, PGPR-3 demonstrated the highest soil phosphatase activity at 0.80. The most substantial enhancement in soil phosphatase activity was observed with the combined application of PGPR-3 and biochar at the rate of 10 tons ha^{-1}, recording an activity level of 0.93.

3.2. *Cadmium content in different plant tissues and its accumulation indices*

The application of biochar and PGPR significantly decreased the Cd content in all plant parts (Table 2). The root Cd content decreased from 9.20 µg g^{-1} in the CK to 5.76 µg g^{-1} in plants treated with 10 tons ha^{-1} of biochar. Similarly, uninoculated plants had a root Cd content of 8.76 µg g^{-1}, while those inoculated with PGPR-3 displayed a root Cd content of 6.52 µg g^{-1}. However, the most substantial reduction in root Cd content occurred with the combined application of 10 tons ha^{-1} biochar and PGPR-3, resulting in a root Cd content of 4.97 µg g^{-1}. The CK plants exhibited the highest shoot Cd content (9.67 µg g^{-1}); however, the application of biochar and PGPR significantly reduced the shoot Cd content. The lowest shoot Cd content, 4.77 µg g^{-1}, was observed with the combined application of biochar (10 tons ha^{-1}) and PGPR-3. Sunflower seeds revealed lower Cd contents than root and shoot. The highest seed Cd content was 3.19 µg g^{-1} and corresponded to the CK plants. Despite the single application of biochar or PGPRs considerably lowered Cd content in seeds, the combined application showed a large and significant reduction in seed Cd content, particularly 10 ton ha^{-1} of biochar and PGPR-3, which recorded a seed Cd content of 0.24 µg g^{-1}.

Sunflowers showed BCF greater than 1 for all the treatments; however, CK plants had the highest BCF of 2.89. Increasing the rate of biochar significantly lowered the BCF, recording the lowest BCF (1.50) at the rate of 10 ton ha^{-1}. Although PGPRs inoculation considerably reduced the BCF compared to CK, the highest reduction corresponded to the PGPR-3. However, the dual combination of PGPR-3 and biochar (10 ton ha^{-1}) revealed the lowest BCF of 1.30. All treatments showed TF values less than 1. Biochar and PGPRs inoculation showed opposite effects on TF. For instance, biochar-treated plants displayed lower TF values than the CK; otherwise, inoculated sunflowers with PGPRs resulted in higher TF values than the CK. While CK plants had a TF value of 0.90, inoculated plants with PGPR-3 showed a TF value of 0.95. Results revealed that biochar (5 ton ha^{-1}) exhibited the lowest TF values, particularly when combined with PGPR-2, recording a TF value of 0.88. Biochar and PGPR treatments recorded BAC greater than 1. Increasing the rate of biochar significantly decreased the values of BAC. The BAC value dropped from 2.23 for CK plants to 1.40 for those that received 10 ton ha^{-1} of biochar. Likewise, inoculated plants with PGPRs showed lower BAC than the CK. The lowest BAC value (1.24) corresponded to the plants that received biochar (10 ton ha^{-1}) and inoculated with PGPR-3.

3.3. *Changes in ROS markers, compatible solutes, and non-enzymatic antioxidants of sunflower under Cd stress*

The stomatal conductance response (mmol H_2O m^{-2} s^{-1}) exhibited significant variations based on the application of biochar and PGPR strains (Fig. 1). The stomatal conductance of CK plants was 38.91, and increased to 46.77 upon PGPR-3 inoculation and further to 50.98 with the addition of biochar (10 tons ha^{-1}). Notably, plants inoculated with PGPR-3 in the presence of biochar (10 tons ha^{-1}) displayed the highest stomatal conductance of 61.20. CK plants had an RWC of 69.0 %, which increased to 81.6 % with PGPR-3 inoculation and further to 86.8 % with biochar (10 tons ha^{-1}). The highest RWC (93.0 %) was observed with the combined application of PGPR-3 and biochar (10 tons ha^{-1}). Conversely, proline content (mg 100 g^{-1} FW) in sunflower leaves significantly decreased due to PGPR and biochar application. The highest proline content was 8.50 in the CK, while those treated with 5 and 10 tons ha^{-1} of biochar had lower proline contents of 6.33 and 5.12, respectively. Additionally, plants inoculated with PGPRs displayed lower proline contents than the CK. The most substantial reduction in proline content was associated with the integrated application of biochar (10 tons ha^{-1}) and PGPR, recording the lowest proline content (4.42). Malondialdehyde (MDA; nmol g^{-1} FW) contents, indicative of lipid peroxidation degree in sunflower leaves, decreased with the application of biochar and PGPRs. The CK plants showed the highest MDA content at 24.6, which dropped to 14.3 upon PGPR-3 inoculation

Table 2

Distribution of cadmium and its accumulation indices in different tissues of sunflower (*Helianthus annuus* L., cv. Sakha 53) irrigated with cadmium-polluted drainage water in the presence of biochar (0, 5, and 10 ton ha^{-1}) and different PGPRs inocula, i.e., PGPR-1 (*Azospirillum brasiliense* SARS 1001+*Bacillus circulans* NCAIM B.02324), PGPR-2 (*Azospirillum brasiliense* SARS 1001+*Pseudomonas koreensis* MG209738), and PGPR-3 (*Azospirillum brasiliense* SARS 1001+*Bacillus circulans* NCAIM B.02324+*Pseudomonas koreensis* MG209738).

		Cadmium content (µg g^{-1})			BCF[‡]	TF[†]	BAC[¥]
		Root	Shoot	Seed			
Biochar	0	9.20±1.19 a	8.56±0.73 a	2.53±0.43 a	2.40±0.31 a	0.94±0.04 a	2.23±0.19 a
	5	7.47±0.73 b	6.80±0.73 b	1.56±0.40 b	1.95±0.19 b	0.91±0.02 a	1.77±0.19 b
	10	5.76±0.67c	5.37±0.52c	0.64±0.38c	1.50±0.17c	0.93±0.02 a	1.40±0.14c
PGPR	CK[Φ]	8.76±1.91a	7.90±1.53 a	2.20±0.85 a	2.28±0.50 a	0.90±0.03 b	2.06±0.40 a
	PGPR-1	7.42±1.37 ab	6.87±1.35 a	1.53±0.81 ab	1.93±0.36 ab	0.93±0.03 ab	1.79±0.35 a
	PGPR-2	7.22±1.35 ab	6.70±1.35 a	1.42±0.81 ab	1.88±0.35 ab	0.93±0.04 ab	1.74±0.35 a
	PGPR-3	6.52±1.37 b	6.17±1.31 a	1.15±0.80 b	1.70±0.36 b	0.95±0.02 a	1.61±0.34 a
Interaction							
0-Biochar	CK	11.11±0.18 a	9.67±0.26 a	3.19±0.07 a	2.89±0.05 a	0.96±0.01 a	2.52±0.07 a
	PGPR-1	8.92±0.06 b	8.47±0.02 b	2.47±0.06 b	2.32±0.01 b	0.96±0.01 ab	2.21±0.00 b
	PGPR-2	8.65±0.08 bc	8.31±0.07 b	2.37±0.04 b	2.25±0.02 bc	0.95±0.01 a	2.16±0.02 b
	PGPR-3	8.13±0.10 d	7.77±0.09c	2.07±0.03c	2.12±0.03 d	0.87±0.00 f	2.02±0.02c
5-Biocahr	CK	8.41±0.03 cd	7.88±0.10c	2.18±0.04c	2.19±0.01 cd	0.94±0.01 abc	2.05±0.03c
	PGPR-1	7.57±0.13 e	6.78±0.12 d	1.51±0.04 d	1.97±0.03 e	0.90±0.02 def	1.76±0.03 d
	PGPR-2	7.45±0.05 e	6.57±0.09 d	1.41±0.05 d	1.94±0.01 e	0.88±0.02 bcd	1.71±0.02 d
	PGPR-3	6.46±0.01 f	5.96±0.03 e	1.14±0.05 e	1.68±0.00 f	0.88±0.00 ef	1.55±0.01 e
10-Biochar	CK	6.74±0.10 f	6.14±0.07 e	1.23±0.05 e	1.75±0.03 f	0.96±0.00 a	1.60±0.02 e
	PGPR-1	5.78±0.09 g	5.37±0.08 f	0.60±0.05 f	1.50±0.02 g	0.93±0.02 abc	1.40±0.02 f
	PGPR-2	5.57±0.02 g	5.20±0.02 f	0.49±0.03 f	1.45±0.00 g	0.93±0.01 abc	1.36±0.01 f
	PGPR-3	4.97±0.10 h	4.77±0.09 g	0.24±0.02 g	1.30±0.02 h	0.91±0.00 cde	1.24±0.02 g

Means in the same column followed by different letters are significant according to the Tukey's test ($P \leq 0.05$). Data are Means ± SD and n = 3. [‡] Biological concentration factor; [†] Translocation factor; [¥] Biological accumulation coefficient; [Φ] Control

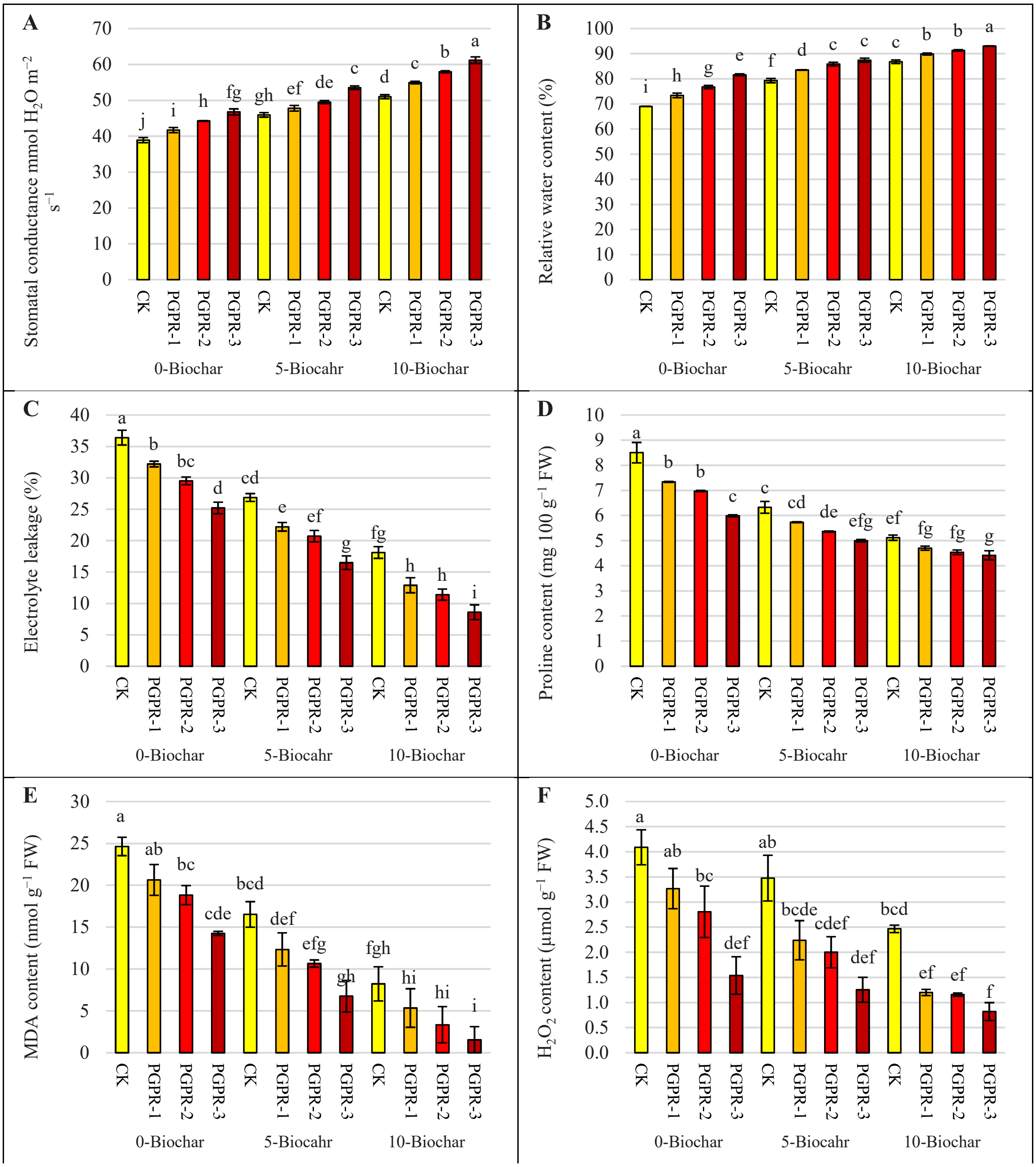

Fig. 1. Physical and chemical alterations, i.e., **A**) stomatal conductance, **B**) relative water content, **C**) electrolyte leakage, **D**) proline content, E) malondialdehyde content, and **F**) hydrogen peroxide content, of sunflower (*Helianthus annuus* L., cv. Sakha 53) irrigated with cadmium-polluted drainage water in the presence of biochar (0, 5, and 10 ton ha^{-1}) and different PGPRs inocula, i.e., PGPR-1 (*Azospirillum brasilense* SARS 1001+*Bacillus circulans* NCAIM B.02324), PGPR-2 (*Azospirillum brasilense* SARS 1001+*Pseudomonas koreensis* MG209738), and PGPR-3 (*Azospirillum brasilense* SARS 1001+*Bacillus circulans* NCAIM B.02324+*Pseudomonas koreensis* MG209738). Different letters on the same bars are significant according to the Tukey's test ($P \leq 0.05$). Data are Means ± SD and n = 3.

and further to 8.2 for those treated with biochar (10 tons ha^{-1}). The lowest MDA content (1.5) was observed with the combined application of biochar (10 tons ha^{-1}) and PGPR-3. Hydrogen peroxide (H_2O_2) content (μmol g^{-1} FW) in sunflower leaves was significantly reduced by biochar and PGPR application. However, the results indicated that PGPRs were more effective in reducing H_2O_2 contents than biochar·H_2O_2 content dropped from 4.09 (for CK) to 1.54 (for those inoculated with PGPR-3). The highest reduction in H_2O_2 corresponded to the dual application of biochar (10 tons ha^{-1}) and PGPR-3, recording the lowest H_2O_2 content (0.82).

3.4. *Efficiency of photosynthetic machinery of sunflower under cadmium stress*

Plants irrigated with Cd-polluted water exhibited a low rate of photosynthesis (μmol m^{-2} sec^{-1}). However, the application of biochar and PGPR significantly alleviated the Cd stress, improving the photosynthetic machinery (Fig. 2). While CK plants had a photosynthetic rate of 7.96, plants treated with biochar (5 tons ha^{-1}) demonstrated a rate of 13.32, which further increased to 16.98 with an increased biochar rate of 10 tons ha^{-1}. Additionally, plants inoculated with PGPR strains displayed higher photosynthetic rates compared to the CK, ranging from 10.40 (for PGPR-1) to 13.82 (for PGPR-3). The highest photosynthetic

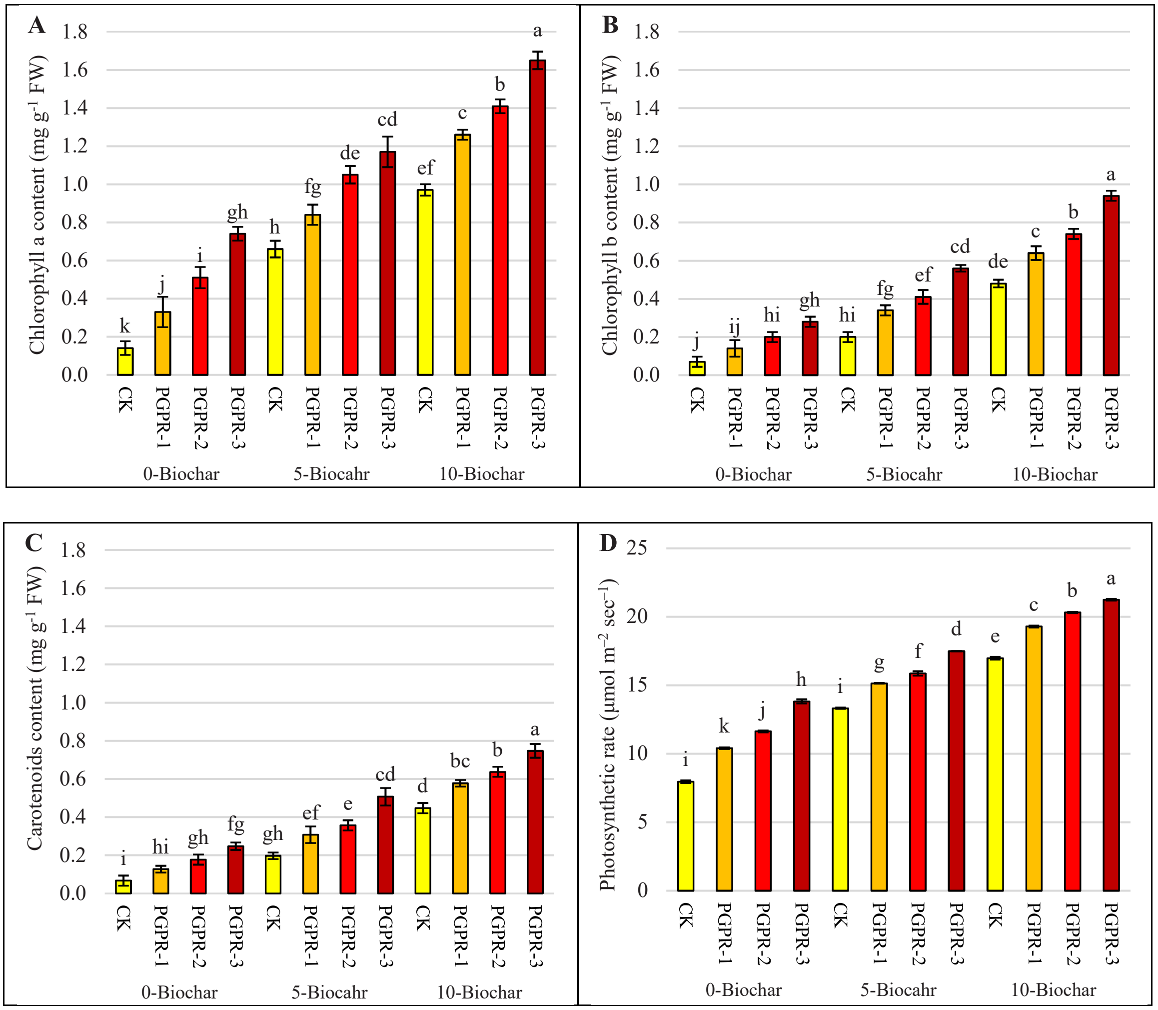

Fig. 2. Efficiency of photosynthetic machinery, i.e., **A**) chl a, **B**) chl b, **C**) carotenoids, and **D**) photosynthetic rate, of sunflower (*Helianthus annuus* L., cv. Sakha 53) irrigated with cadmium-polluted drainage water in the presence of biochar (0, 5, and 10 ton ha^{-1}) and different PGPRs inocula, i.e., PGPR-1 (*Azospirillum brasilense* SARS 1001+*Bacillus circulans* NCAIM B.02324), PGPR-2 (*Azospirillum brasilense* SARS 1001+*Pseudomonas koreensis* MG209738), and PGPR-3 (*Azospirillum brasilense* SARS 1001+*Bacillus circulans* NCAIM B.02324 + *Pseudomonas koreensis* MG209738). Different letters on the same bars are significant according to the Tukey's test ($P \leq 0.05$). Data are Means ± SD and n = 3.

rate (21.24) was observed with the combined application of biochar (10 tons ha^{-1}) and PGPR. Similarly, CK plants showed low contents of chlorophyll a (chl a), chlorophyll *b* (chl b), and carotenoids, whereas plants treated with biochar and PGPRs exhibited elevated levels of photosynthetic pigments. CK plants had 0.14, 0.07, and 0.07 mg g^{-1} FW of chl a, chl b, and total carotenoids, respectively. In contrast, plants treated with 10 tons ha^{-1} of biochar had contents of 0.97, 0.48, and 0.45 mg g^{-1} FW of chl a, chl b, and carotenoids, respectively. Plants subjected to the combined application of biochar (10 tons ha^{-1}) and PGPR-3 displayed the highest contents of chl a, chl b, and carotenoids at 1.65, 0.94, and 0.75 mg g^{-1} FW, respectively.

3.5. Efficiency of enzymatic antioxidant capacity of sunflower plants

Biochar application and PGPR significantly increased the activities of antioxidant enzymes, i.e., CAT (μmol H_2O_2 g^{-1} FW min^{-1}), POD (μM tetraguaiacol g^{-1} FW min^{-1}), SOD (μmol H_2O_2 g^{-1} FW min^{-1}), and PPO (μM tetraguaiacol g^{-1} FW min^{-1}) (Fig. 3). The lowest CAT activity (40.7) corresponded to the CK. Inoculated plants with PGPRs showed higher CAT activity than the CK; however, PGPR-3 displayed the highest CAT activity (46.3) among PGPRs. The combined application of biochar (10 ton ha^{-1}) and PGPR-3 exhibited the CAT activity of 73.5. Similarly, CK plants displayed a POD activity of 20.8, which increased to 23.7 (for PGPR-3) and 27.2 (for 10 ton biochar ha^{-1}). Inoculation of sunflower plants with PGPRs, especially in conjugation with 10 ton biochar ha^{-1}, revealed the highest POD activities. The highest POD activity (37.3) corresponded to the dual application of biochar (10 ton ha^{-1}) and PGPR. The SOD activity almost doubled upon treating plants with biochar (10 ton ha^{-1}) and PGPR-3, recording activity of 43.7, while CK plants had a SOD activity of 27.2. However, all treated plants with either PGPRs, biochar, or their combinations displayed higher SOD activities than the CK. The PGPR-1 and PGPR-2 revealed similar activities, while PGPR-3 documented higher SOD activities. Also, applying biochar resulted in higher SOD activities than the CK. Similarly, PPO activity exhibited a consistent response to the application of biochar and PGPR, mirroring the patterns observed for other antioxidant enzymes such as CAT, POD, and SOD. A twofold increase in PPO activity was observed when plants were inoculated with PGPR-3 in conjunction with biochar (10 tons ha^{-1}) compared to the CK. Plants subjected to the combined application of PGPR-3 and biochar (10 tons ha^{-1}) displayed a PPO activity of 1.05, whereas CK plants had a PPO activity of 0.46. Notably, the singular application of either PGPR or biochar increased PPO activity compared to the CK. However, the most substantial increase in PPO activity was observed with the combined application of biochar (10 tons ha^{-1}) and PGPRs.

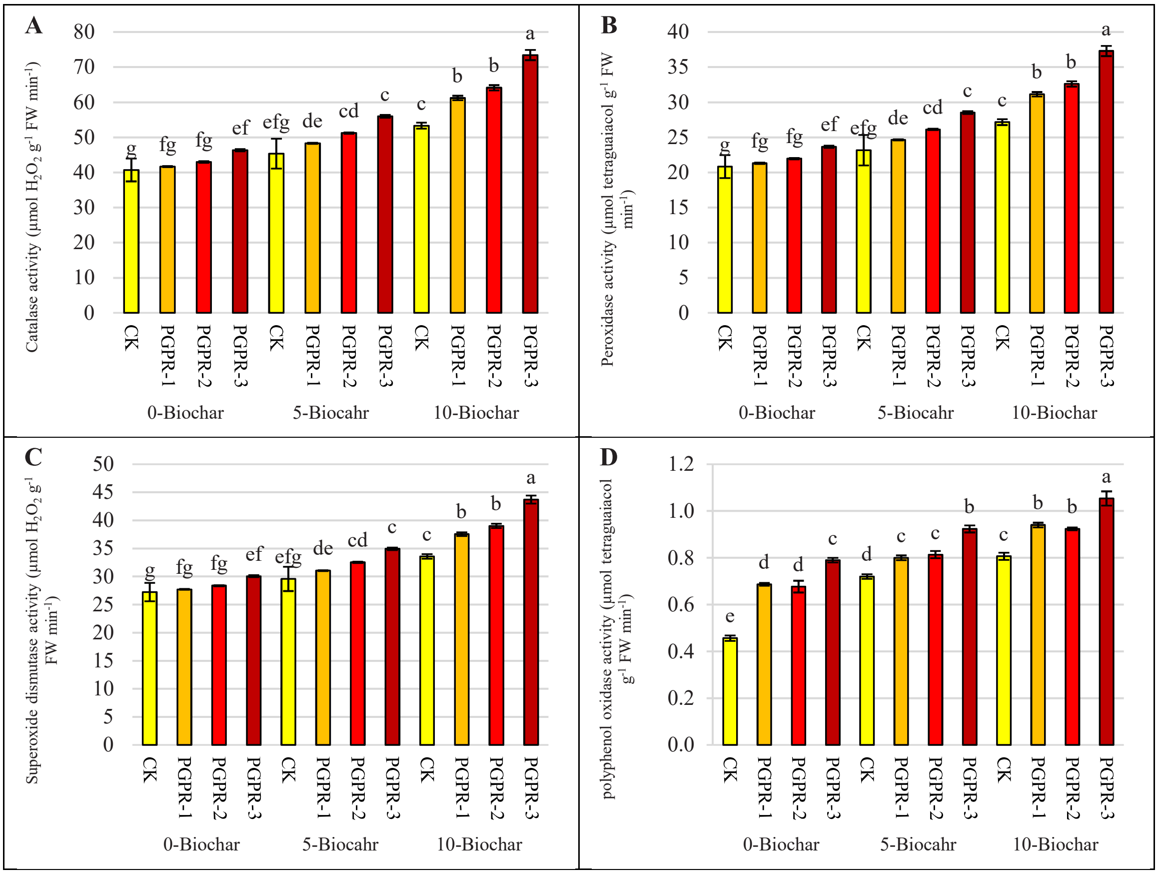

Fig. 3. Efficiency of enzymatic-antioxidant capacity, i.e., **A**) catalase, **B**) peroxidase, **C**) superoxide dismutase, and **D**) polyphenol oxidase, of sunflower (*Helianthus annuus* L., cv. Sakha 53) irrigated with cadmium-polluted drainage water in the presence of biochar (0, 5, and 10 ton ha^{-1}) and different PGPRs inocula, i.e., PGPR-1 (*Azospirillum brasilense* SARS 1001+*Bacillus circulans* NCAIM B.02324), PGPR-2 (*Azospirillum brasilense* SARS 1001+*Pseudomonas koreensis* MG209738), and PGPR-3 (*Azospirillum brasilense* SARS 1001+*Bacillus circulans* NCAIM B.02324+*Pseudomonas koreensis* MG209738). Different letters on the same bars are significant according to the Tukey's test ($P \leq 0.05$). Data are Means ± SD and $n = 3$.

Table 3

Characteristics of seed and oil yields of sunflower (*Helianthus annuus* L., cv. Sakha 53) irrigated with cadmium-polluted drainage water in the presence of biochar (0, 5, and 10 ton ha^{-1}) and different PGPRs inocula, i.e., PGPR-1 (*Azospirillum brasilense* SARS 1001+*Bacillus circulans* NCAIM B.02324), PGPR-2 (*Azospirillum brasilense* SARS 1001+*Pseudomonas koreensis* MG209738), and PGPR-3 (*Azospirillum brasilense* SARS 1001+*Bacillus circulans* NCAIM B.02324+*Pseudomonas koreensis* MG209738).

		100-seed weight	Seed yield	Oil percentage	Oil yield
		(g)	(kg ha^{-1})	(%)	(kg ha^{-1})
Biochar	0	5.58±0.72c	1801±172c	32.2±3.55c	468±45c
	5	7.16±0.81 b	2207±199 b	40.5±5.02 b	574±52 b
	10	8.78±0.80 a	2581±172 a	46.7±2.51 a	671±45 a
PGPR	CK$^{\Phi}$	6.08±1.38 b	1949±325 b	35.0±6.85 b	507±85 b
	PGPR-1	7.20±1.29 ab	2199±350 ab	39.5±6.95 ab	572±91 ab
	PGPR-2	7.30±1.44 ab	2208±356 ab	40.4±6.21 ab	574±92 ab
	PGPR-3	8.11±1.48 a	2430±328 a	44.4±6.02 a	632±85 a
Interaction					
0-Biochar	CK	4.52±0.30 h	1586±34 i	27.3±1.29 g	412±9 i
	PGPR-1	5.73±0.25 g	1788±18 h	32.3±0.92 f	465±5 h
	PGPR-2	5.71±0.17 g	1786±32 h	32.8±0.32 ef	464±8 h
	PGPR-3	6.35±0.05 f	2042±49 f	36.6±1.12 de	531±13 f
5-Biocahr	CK	6.04±0.17 fg	1927±40 g	34.8±0.63 def	501±10 g
	PGPR-1	7.19±0.13 e	2213±31 e	38.8±4.27 cd	575±8 e
	PGPR-2	7.19±0.15 e	2235±49 de	41.5±0.77 c	581±13 de
	PGPR-3	8.21±0.17 cd	2455±41 c	47.0±1.23 ab	638±11 c
10-Biochar	CK	7.68±0.21 de	2333±32 d	43.0±0.28 bc	607±8 d
	PGPR-1	8.67±0.31 bc	2595±16 b	47.5±0.54 a	675±4 b
	PGPR-2	9.01±0.22 b	2603±17 b	46.9±0.08 ab	677±5 b
	PGPR-3	9.75±0.18 a	2793±22 a	49.6±0.20 a	726±6 a

Means in the same column followed by different letters are significant according to the Tukey's test ($P \leq 0.05$). Data are Means ± SD and $n = 3$. $^{\Phi}$ Control

3.6. Characteristics of seed and oil yields

The application of biochar and PGPR resulted in a significant increase in seed and oil yields for sunflowers irrigated with Cd-polluted water (Table 3). The 100-seed weight increased significantly from 5.58 g for CK plants to 8.78 g for plants treated with biochar (10 tons ha^{-1}). Inoculated plants with PGPRs also exhibited greater 100-seed weights than the CK, with PGPR-3 achieving the highest at 8.11 g. The most notable enhancement in 100-seed weight occurred with the combined application of PGPR-3 and biochar (10 tons ha^{-1}), recording the highest 100-seed weight of 9.75 g. The seed yield of sunflowers significantly increased from 1801 kg ha^{-1} for CK plants to 2581 kg ha^{-1} with the addition of biochar at a rate of 10 tons ha^{-1}. Inoculation with PGPRs, particularly PGPR-3, resulted in a seed yield of 2430 kg ha^{-1}. All inoculated plants with PGPRs achieved higher seed yields than the CK. Plants treated with biochar (10 tons ha^{-1}) and PGPR-3 exhibited the highest seed yield of 2793 kg ha^{-1}. Similarly, biochar application enhanced the oil yield of sunflowers, with plants treated with 10 tons ha^{-1} of biochar reaching an oil yield of 671 kg ha^{-1}, compared to 468 kg ha^{-1} for CK plants. The oil yield increased from 507 kg ha^{-1} for uninoculated plants to 632 kg ha^{-1} for those inoculated with PGPR-3. However, the combined application of PGPR-3 and biochar (10 tons ha^{-1}) resulted in the highest oil yield of 726 kg ha^{-1}. The oil percentage of CK plants was 32.2 % and increased to 46.7 % with the application of biochar at a rate of 10 tons ha^{-1}. Inoculated plants with PGPR-3 had an oil percentage of 44.4 %, while uninoculated plants (CK) displayed an oil percentage of 35.0 %. A significant improvement in the oil percentage of sunflower seeds was observed with the dual application of PGPRs and biochar at a rate of 10 tons ha^{-1}. The highest recorded oil percentage (49.6 %) was associated with the application of biochar (10 tons ha^{-1}) and PGPR.

4. Discussion

The majority of biochars tend to exhibit alkaline properties (Jiang et al., 2012). Elevated pyrolysis temperatures result in increased nutrient content, specific surface area, and pH of biochar (Kloss et al., 2012); however, this process also eliminates acidic functional groups, leading to a more basic nature of biochar (Ahmad et al., 2012). Consequently, there is a scarcity of literature focusing on the examination of biochar in alkaline soils. Nonetheless, it is important to recognize that biochar may offer several advantages in alkaline soils, including pH buffering, augmentation of cation exchange capacity, enhancement of water retention, stimulation of microbial activity, and amplification of adsorption capacities for nitrous oxide (N_2O), nitric oxide (NO), and ammonia (NH_3) (Liu et al., 2022). Therefore, the objective of this study is to underscore the potential benefits of biochar in saline-alkali soil, particularly when combined with PGPR.

4.1. Impacts of Cd-polluted irrigation water on soil properties

Irrigating with drainage water containing Cd can adversely affect the physical, chemical, and biochemical properties of the soil, influencing plant growth and overall soil health. The accumulation of Cd has implications for the aggregation of soil particles, resulting in reduced soil porosity and increased bulk density. Consequently, the soil's water retention capacity diminishes, and the infiltration of water and air is hindered (Huang et al., 2022). While saline soils typically exhibit high pH levels, Cd can induce soil acidification by interacting with soil particles and displacing other cations, including calcium and magnesium. This process contributes to an increase in soil acidity (Kicińska et al., 2022). Saline soils, already containing elevated levels of soluble salts, may experience exacerbated salinity issues with the addition of Cd. Cd interacts with soil salts, enhancing their mobility and leading to an increase in soil salinity (Abbas et al., 2017). In the current study, irrigation with Cd-polluted water resulted in a decrease in soil pH from 8.22 (before the experiment) to 8.18 (at the end of the experiment) and an increase in electrical conductivity (EC) by 6.1 % (Table 1). However, a study focusing on the impact of sewage water irrigation on soil under cotton-wheat rotation for over 20 years indicated an increase in soil pH by 0.3 % and in EC by 0.7 % (Abbas et al., 2017). Saline soils typically exhibit poor drainage, diminishing the leaching of Cd from the soil profile. Also, the diminished soil microbial activity restricts the biotransformation and degradation of Cd, contributing to its accumulation in the soil (Chen et al., 2014). In this study, the irrigation of sunflowers with Cd-polluted water resulted in a 10.4 % increase in the content of available Cd in the soil. However, higher Cd concentrations in irrigation water will lead to a more substantial increase in soil Cd content. The irrigation water in this study had a Cd content of 0.094 mg L^{-1}, exceeding background values (Ayers and Westcot, 1985). Moreover, the physical and chemical properties of the soil, such as pH, organic matter (SOM), and clay content, can influence the adsorption and retention of Cd. Soils with low SOM and high clay content may possess higher Cd retention capacities (Alloway, 2013). Our soil had low SOM (11.2 g kg^{-1}) and was classified as clayey soil, hence resulting in an increased content of available Cd in the soil following irrigation with Cd-polluted water. This effect is expected to persist based on the frequency and volume of irrigation (Hu et al., 2013). Cadmium contamination can decrease soil microbial diversity and biomass, disrupting soil ecosystem functions and diminishing the natural remediation capacity of the soil. Reduced microbial activity leads to a decline in the decomposition of organic matter and nutrient cycling, consequently lowering rates of soil respiration (Reber, 1989). Soil respiration is closely associated with the decomposition of organic matter by soil microorganisms. The experimental soil in this study had a low SOM content of 11.2 g kg^{-1}, resulting in the CK plots displaying the lowest soil respiration at 20.5 mg CO_2 100 g^{-1} soil 24 h^{-1} (Table 1). A study investigating the effect of Cd (1.5–6.0 mg kg^{-1}) on soil microbial activities reported a reduction in soil respiration attributed to Cd toxicity (Chen et al., 2014).

Cadmium toxicity has the potential to hinder dehydrogenase enzyme activity (Yeboah et al., 2021), indicating a decrease in microbial metabolic processes within the soil. The reduction in dehydrogenase activity has adverse effects on soil respiration and nutrient cycling, contributing to the degradation of soil health (Zhang et al., 2010). Nitrogenase, an enzyme responsible for nitrogen fixation, facilitates the conversion of atmospheric nitrogen (N_2) into ammonia (NH_3), a nutrient utilized by plants (Ao et al., 2023). Cadmium is known to impede the activity of nitrogenase in the soil (Irfan et al., 2013). Irrigation of crops with drainage water containing Cd can result in the accumulation of the metal in the soil, leading to diminished nitrogenase activity. This inhibition restricts the availability of fixed nitrogen for crops, causing nitrogen deficiency and impeding plant growth and development. Phosphatase play a pivotal role in releasing phosphorus from organic matter and converting it into forms accessible to plants (Margalef et al., 2017). Cadmium contamination in soil can disrupt phosphatase activity. An increase in Cd concentration in soil from 0.3 to 30 mg kg^{-1} resulted in a reduction in acid phosphatase activity by 15.2–32.6 % (Zheng et al., 2019). Cadmium ions can bind to phosphatase, altering their structure and diminishing their catalytic efficiency. In our study, CK plots exhibited the lowest soil dehydrogenase, nitrogenase, and phosphatase activities compared to those inoculated with different PGPRs or receiving biochar.

4.2. Improvement in soil characteristics upon biochar and PGPR co-application

The findings of this study indicate a slight decrease in soil pH with the addition of biochar; however, the concurrent application of biochar and PGPR-3 resulted in a more pronounced decrease in soil pH (Table 1). Biochar has the ability to buffer soil pH by absorbing and releasing hydrogen ions as needed. Furthermore, altering soil microbial activity, nutrient availability, and organic matter decomposition by biochar can impact soil acidity levels (Jeffery et al., 2017). Maintaining a stable pH is crucial for creating a favorable environment for soil microorganisms and ensuring nutrient availability (Alkharabsheh et al., 2021). In the current study, the pH of the experimental soil was 8.22, while the pH of the biochar was 7.60; hence, a decrease in soil pH was noted upon the addition of biochar. Inoculating sunflower seeds with different PGPRs in this study resulted in lower soil pH compared to the CK due to the production of organic acids (De Andrade et al., 2023; Upadhyay et al., 2022); however, the combined application of biochar and PGPRs led to even lower soil pH values (Table 1). Similarly, the pH of soil cultivated with raspberry and inoculated with two *Bacillus* variants was lower than the control by 14.9 % (Orhan et al., 2006).

The application of biochar and PGPR-3 resulted in a considerable reduction of available Cd content to 0.41 mg kg^{-1} compared to 1.06 mg kg^{-1} for the CK plots. Likewise, soil received biochar at rates of 1–2 % w/w showed a reduction of 5.11–35.14 % of extractable Cd content in soil polluted with 30 mg Cd kg^{-1} (Yao et al., 2021). The combined application of biochar and PGPR represents a promising approach for detoxifying Cd contamination in saline-alkali soil. Firstly, biochar enhances soil microbial activity and diversity, fostering a favorable environment for PGPR colonization and activity. Subsequently, PGPR can biodegrade Cd or immobilize it through complexation or precipitation reactions, thereby diminishing its bioavailability to plants (Sarfraz et al., 2019). In the present study, the co-application of PGPR-3 and biochar at the rate of 10 ton ha^{-1} resulted in a 137.1 % increase in soil respiration (Table 1). Additionally, PGPR established symbiotic relationships with plant roots in the rhizosphere, promoting root exudation that chelates Cd ions and facilitates their immobilization in the soil. This reduces Cd uptake by plants and mitigates its toxic effects (Sarfraz et al., 2019). The observed promotion of bacterial α-diversity is an indicator for the established symbiotic relationship between soil rhizosphere microorganisms and plant roots (Pariyar et al., 2020). The application of biochar at a rate of 1 % w/w demonstrated a significant promotional effect on bacterial α-diversity. Furthermore, PGPR can stimulate plant growth and enhance plant health, augmenting plant tolerance to Cd stress. Biochar-amended soils provide a stable habitat for PGPR, improving nutrient availability and supporting plant growth and Cd tolerance (Ren et al., 2022). Moreover, biochar's high surface area and porous structure enable it to adsorb and immobilize Cd ions in the soil, thereby reducing their bioavailability to plants and detoxifying Cd-contaminated soil (Anwar et al., 2024). Biochar amendments also exert indirect effects on soil properties, enhancing physicochemical attributes such as pH, cation exchange capacity, and water retention. These improvements influence Cd mobility and availability, with PGPR-assisted biochar application further enhancing these soil properties and contributing to Cd immobilization and detoxification (Sarfraz et al., 2019). Finally, PGPR possess enzymatic machinery capable of degrading Cd or transforming it into less toxic forms. This microbial activity, facilitated by biochar, accelerates the biodegradation of Cd and reduces its environmental impact (Ren et al., 2022).

The application of biochar resulted in enhanced activities of soil invertase, urease, polyphenol oxidase, and catalase, consistent with the findings reported by (Buchkowski et al., 2019). Similarly, in our current investigation, soil dehydrogenase, nitrogenase, and phosphatase activities exhibited increases of 79.9 %, 217.4 %, and 85.4 %, respectively, with the application of 10 ton ha^{-1} of biochar. Moreover, when plants were inoculated with PGPR-3 and received 10 ton ha^{-1} of biochar, these activities increased even further, by 129.6 %, 326.1 %, and 126.8 %, respectively (Table 1). In another study, the activities of soil phosphatase, urease, sucrose, and catalase increased by 23.2 %, 27.9 %, 63.3 %, and 50.3 %, respectively, with the addition of 2 % biochar compared to the control (Jiang et al., 2021). The addition of biochar has been linked to an increase in soil carbon content (Case et al., 2014), leading to an elevated soil C/N ratio and enhanced microbial activities, including enzyme production. In an experiment involving antiseptic bulk soil, the inoculation of *Bacillus subtilis* (BS) and *Pseudomonas fluorescens* (PF) increased soil urease activity by 90 % and 70 %, respectively. This increase was closely associated with improved activity and populations of soil microorganisms, with *B. subtilis* showing higher numbers (0.78–0.97CFU mL^{-1}) in the soil than *P. fluorescens* (0.55–0.79 CFU mL^{-1}) (Ng et al., 2022).

4.3. Effect of combined application of biochar and PGPR on Cd uptake and translocation in sunflowers

Despite its high mobility in the soil solution and easy access to root cells, the translocation of Cd to the aboveground parts of most plant species is limited. Cd particles are primarily retained in the roots of plants, with only a small portion being transported to the aerial parts of the plant. The order of Cd accumulation in plant parts typically follows this sequence: roots > leaves > fruits > grains (Kubier et al., 2019). Our results align with these findings, where sunflower roots exhibited the highest Cd content, followed by shoots, and seeds had the lowest values (Table 2). Similarly, in soybean plants, about 98 % of the total accumulated Cd was found in roots (Sarwar et al., 2010).

Biochar is a solution to enhance soil properties when irrigated with Cd-polluted water, employing various mechanisms to alleviate the adverse effects of Cd on both soil and plants. Due to its high surface area and porous structure, biochar can adsorb and retain heavy metals, such as Cd, by binding with hydroxides, carbonates, and organic substances. When introduced into salt-affected soil, biochar served as a sink for Cd^{2+}, diminishing their bioavailability to plants (Meng et al., 2022). In a pot experiment conducted on Cd-polluted saline-alkali soil, biochar addition led to a reduction in Cd accumulation in plant tissues and lowered residual Cd concentrations. Additionally, biochar effectively decreased the mobility and availability of residual Cd over an extended period, offering protection against vertical leaching and safeguarding underground water from Cd contamination (Sun et al., 2020). Moreover, utilization of different organic substances decreased Cd uptake by rice and wheat plants when they were irrigated with sewage water, especially rice husk biochar (Shaghaleh et al., 2024). The application of rice residues-based biochar, ranging from 1 % to 5 % w/w, significantly reduced Cd accumulation in plant tissues, concurrently decreasing Cd translocation towards grains in a pot experiment (Abbas et al., 2017). Furthermore, the use of rice straw-based biochar at a 2 % rate in Cd-polluted soil ($>$ 5 mg kg^{-1}) resulted in a 71.1 % reduction in Cd content in sunflower shoots and a 67.2 % reduction in Cd content in roots (Bashir et al., 2021). In another study, the application of biochar reduced Cd content in cotton roots grown in Cd-polluted soil (4 mg kg^{-1}) by 13.78 % (Zhu et al., 2022). On the other hand, nanoparticles such as zinc oxide nanoparticles applied at a rate of 300 mg/kg significantly minimized Cd absorption by wheat plants (Usman et al., 2023).

In the current investigation, the application of biochar at rates of 10 ton ha^{-1} significantly decreased Cd content in the roots, shoots, and seeds of sunflowers by 39.3 %, 36.5 %, and 61.4 %, respectively. Notably, higher reductions in Cd contents across sunflower parts, including roots (55.3 %), shoots (50.7 %), and seeds (92.5 %), were observed with the co-application of biochar (10 ton ha^{-1}) and PGPR-3 (Table 2). Our findings also indicated minimal translocation of Cd from roots to aboveground parts. Conversely, contrary results were presented by (Yao et al., 2021) in their tobacco plant study, reporting higher Cd accumulation in leaves than in the root system, signifying elevated Cd transportation from roots to leaves. However, they noted that older leaves exhibited 2–4 times higher Cd contents than younger leaves, suggesting an adaptive process in which tobacco plants shield younger leaves from Cd toxicity (Dguimi et al., 2019). Biochar-amended and PGPR-inoculated plots in our study exhibited lower accumulation indices (BCF, TF, and BAC) of Cd in sunflowers compared to the CK plots. The combined application of biochar (10 ton ha^{-1}) and PGPR-3 demonstrated higher reductions in BCF, TF, and BAC, registering 55.0 %, 5.2 %, and 50.8 %, respectively (Table 2). The positive impact of biochar and PGPR application on reducing Cd phytoavailability may be attributed to the enhancement of soil properties, such as fertility, structure, and microbial activity, leading to the immobilization of toxic metals like Cd through complexation. This process, coupled with the induction of plant growth and stimulation of root exudates, contributes to the fixation of toxic metals (Elsayed et al., 2020; Vinci et al., 2018). Additionally, PGPR can stimulate the expression of metal detoxification genes in plants, playing a crucial role in the plant's defense against heavy metal toxicity. The up-regulation of these genes enhances the plant's efficiency in detoxifying Cd, mitigating its adverse effects on both the plant and the soil (Burd et al., 2000). *Bacillus* and *Pseudomonas* species are among PGPR with high capacity to detoxify several contaminants such as Ni, Pb, Cu, Cd, and Cr (El-Nahrawy et al., 2019; Karimpour et al., 2018). Five days after inoculation, *Saccharomyces cerevisiae* L. and *B. subtilis* L. absorbed 69.6 % and 75.8 % of total Cd concentration in contaminated growth medium (Imam, 2016).

4.4. Effect of biochar and PGPR co-application on physicochemical traits of sunflowers under Cd stress

Cadmium stress disrupts root ultrastructure, impairs plant physicochemical traits, and diminishes nutrient uptake, ultimately hindering plant growth and reducing biomass (Ali et al., 2018; Bernhoft, 2013). In our study, sunflowers subjected to CK exhibited elevated EL, proline, MDA, and H_2O_2 contents, along with reduced stomatal conductance and RWC (Fig. 1). However, the co-application of PGPR-3 and biochar (10 ton ha^{-1}) increased stomatal conductance and RWC by 57.3 % and 34.8 %, respectively, while it decreased EL, proline, MDA, and H_2O_2 contents by 76.4 %, 48.0 %, 93.9 %, and 80.0 %, respectively.

Our results align with prior findings. For instance, treating *Mentha piperita* with 160 g kg^{-1} biochar alleviated Cd stress (10 mg kg^{-1}) through increasing stomatal conductance by 80 % compared to the control (Jiang et al., 2022). In another study, the combination of Cd-polluted water and drought stress (35 % of WHC) negatively impacted wheat growth, reducing stomatal conductance by 36 %. However, biochar application (5 % w/w) increased stomatal conductance by 70 % (Abbas et al., 2018). Cd-stressed (100 μM) tobacco plants exhibited higher proline content, aiding in plant cell growth and expansion (Islam et al., 2009). Additionally, Cd stress (0.25–4 mg kg^{-1}) increased MDA and EL in cotton plant roots; nevertheless, biochar and biofertilizers (mainly *Bacillus* spp.) significantly reduced MDA by 29.57 % and 37.95 %, and EL by 14.72 % and 16.80 %, respectively (Zhu et al., 2022). Drought-stressed (35 % WHC) wheat plants in Cd-polluted soil displayed increased H_2O_2 content and EL, which decreased by 27 % and 36 %, respectively, upon the addition of biochar (5 % w/w) in Cd-polluted soil (Abbas et al., 2018). Similarly, inoculation of Cd-stressed wheat with *Bacillus siamensis* resulted in lower MDA content, improved membrane stability, and enhanced photosynthesis (Awan et al., 2020). Additionally, maize and wheat inoculated with PGPR (i.e., Klebsiella, Stenotrophomonas, Bacillus, and Serratia) under Cd stress (40 and 80 mg kg^{-1}) showed increased RWC and biomass yield, along with reduced Cd uptake and EL (Ahmad et al., 2014). *Pseudomonas aeruginosa* enhanced Cd tolerance in contaminated soil by releasing antibiotics like erythromycin, penicillin, amoxicillin, cephalexin, and streptomycin (Muneer et al., 2016). The co-application of biochar and PGPR enhances soil water retention capacity, improving water uptake by plants and leading to higher RWC in plant tissues. This signifies an improved water balance and reduced water stress. Moreover, the joint application of biochar and PGPR strengthens the plant cell membrane, reducing its permeability and decreasing EL, mitigating cellular damage caused by Cd stress. PGPR induces proline synthesis, acting as an osmoprotectant to help plants cope with environmental stress. Biochar enhances this response by providing a better environment for PGPR colonization and activity. Together, biochar and PGPR collectively minimize oxidative stress in plants, resulting in reduced lipid peroxidation and lower MDA levels, indicating less cellular damage from reactive oxygen species (ROS). This integrated approach holds great promise for enhancing plant resilience and productivity under stress conditions (Zainab et al., 2021; Zhang et al., 2019).

4.5. Effect of biochar and PGPR co-application on photosynthesis efficiency of sunflowers under Cd stress

The photosynthetic rate and chlorophyll contents are crucial indicators for assessing the plant's response to adverse conditions, particularly in terms of photosynthetic physiology. Cd stress has been shown to significantly decrease photosynthetic intensity in *Mentha piperita*, impacting gas exchange parameters and chlorophyll content (Jiang et al., 2022). Similarly, the irrigation of sunflower plants with Cd-polluted water in our study negatively influenced the photosynthetic machinery, resulting in reduced content of photosynthetic pigments and photosynthetic rates (Fig. 2). This adverse impact on photosynthesis is attributed to Cd's ability to enhance enzymatic degradation, hinder chlorophyll synthesis, and restrict the opening and closing of stomata in plant leaves (Haider et al., 2021). In contrast, the treatment of *Mentha piperita* with 160 g kg^{-1} biochar alleviated the adverse effects of Cd stress (10 mg kg^{-1}) by significantly increasing the photosynthetic rate and content of photosynthetic pigments by 113 % and 39 %, respectively, compared to the control (Jiang et al., 2022). The most notable improvement in the photosynthetic machinery was observed with the co-application of 10 ton biochar ha^{-1} and PGPR-3. This combination increased the contents of chlorophyll a, chlorophyll *b*, carotenoids, and photosynthetic rate by 12-, 13-, 10-, and 3-fold, respectively, compared to the control (Fig. 2). The positive effects of biochar treatment can be attributed to various factors. Firstly, biochar acts as a Cd sink in the soil, preventing its uptake by plants. Secondly, it protects the plant's chlorophyll and photosynthetic apparatus from damage induced by Cd. Additionally, biochar helps alleviate the negative impact of Cd on leaf stomata, thereby enhancing photosynthetic efficiency in plants, even in the presence of Cd contamination (Haider et al., 2022).

4.6. Effect of biochar and PGPR co-application on enzymatic defense system of sunflowers under Cd stress

Our study unveiled a disruption in the balance between ROS production and scavenging in sunflowers under Cd stress, resulting in substantial alterations in the plant's metabolism. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO) are recognized as protective enzymes that shield the cell membrane from disruptions caused by Cd stress (Abbas et al., 2017). As illustrated in Fig. 3, the activities of SOD, CAT, POD, and PPO in sunflower leaves decreased in the CK upon irrigation with Cd-polluted water. Nevertheless, the addition of biochar and PGPR significantly elevated the activities of these antioxidant enzymes. Following treatment with biochar (10 ton ha^{-1}) and PGPR-3, the activities of SOD, CAT, POD, and PPO increased by 60.5 %, 80.4 %, 79.0 %, and 130.7 %, respectively (Fig. 3). The enhanced adsorption capacity of the soil for heavy metals, facilitated by the addition of biochar, mitigated the presence of Cd in plant tissues (Table 2), leading to a reduction in damage to the plant's plasma membrane. Under Cd stress (4 mg kg^{-1}), the activities of SOD and CAT in the roots of cotton plants were reduced by 6.53 % and 31.04 %, respectively. However, independent applications of biochar and biofertilizers, primarily consisting of *Bacillus* spp., increased the activities of SOD by 4.72 % and 5.90 %, respectively, and CAT by 67.10 % and 88.28 %, respectively (Zhu et al., 2022). Exposure of wheat plants to Cd and drought stress (35 % WHC) significantly reduced the activities of SOD and CAT, while elevating POD activity. However, the addition of biochar (5 % w/w) reversed the activities of antioxidant enzymes, increasing SOD and CAT activities while decreasing POD activity (Abbas et al., 2018). Additionally, inoculation of Cd-stressed wheat with *Bacillus siamensis* resulted in higher activities of SOD and CAT, enhancing overall yield (Awan et al., 2020). In a separate study, the addition of biochar at a rate of 160 g kg^{-1} significantly decreased the activities of SOD, CAT, POD, and PPO enzymes by 26 %, 61 %, 67 %, and 46 %, respectively (Jiang et al., 2022). Similarly, activities of SOD, POD, and CAT in sunflower leaves exposed to Cd stress (5 mg kg^{-1}) showed higher values than the control; however, treatment of the soil with 2 % w/w rice straw-based biochar resulted in decreased activities of these enzymes by 49.2 %, 40.5 %, and 46.5 %, respectively (Bashir et al., 2021).

4.7. Impact of combined application of biochar and PGPR on seed and oil yields of sunflowers under Cd stress

The CK plants displayed the lowest seed and oil yields, as well as oil percentage due to Cd contamination (Table 3). In contrast, the combined application of biochar (10 ton ha^{-1}) and PGPR-3 significantly increased the 100-seed weight, seed yield, oil yield, and oil percentage by 115.6 %, 76.1 %, 76.2 %, and 81.8 %, respectively (Table 3). Similarly, a study on sunflower growth and yield in Cd-polluted soil revealed that rice straw-based biochar application (2 % w/w) increased nutrient uptake, resulting in enhanced sunflower achene, flower diameter, and 1000 achene weight compared to the control (Bashir et al., 2021). Additionally, the application of biochar at a rate of 5 % w/w significantly improved growth characteristics and increased grain yield (by 106 %) of wheat plants grown on Cd-polluted soil (2.86 mg kg^{-1}) and exposed to drought stress (35 % WHC) (Abbas et al., 2017). Biochar application has the potential to stimulate plant growth and root development. Its presence in the soil promotes root elongation and branching, increasing the root surface area, which facilitates nutrient and water uptake by plants, even under Cd-stressed conditions (Haider et al., 2022). Moreover, biochar exhibits a long-term residual effect in the soil, persisting for years and continuously benefiting soil properties, thereby mitigating the adverse impacts of Cd. This characteristic makes biochar a sustainable and effective strategy for long-term soil improvement (Zhang et al., 2021). PGPR plays a crucial role in nutrient cycling and solubilization. These beneficial bacteria produce organic acids, enzymes (such as phosphatases), and siderophores that can solubilize essential nutrients, including phosphorus, iron, and other micronutrients. In Cd-polluted soils, where nutrient availability may be limited due to metal toxicity, PGPR facilitate the release of nutrients, making them more accessible to plants (De Andrade et al., 2023). PGPR can directly or indirectly contribute to the detoxification of Cd in the soil. Some bacteria possess the ability to enzymatically convert or sequester Cd, thereby reducing its toxicity to plants. Additionally, PGPR can stimulate the expression of stress-related genes in plants, enhancing tolerance against Cd stress (El-Nahrawy et al., 2019).

5. Conclusion

The combined use of biochar and PGPR holds significant promise for mitigating the adverse effects of irrigating sunflowers with Cd-contaminated water in saline-alkali soils. This integrated approach offers multiple benefits by enhancing soil properties and improving plant physiological and productivity parameters. Biochar contributes to better soil characteristics, such as water retention, soil pH balance, and nutrient availability, which are crucial for decreasing Cd availability in the soil. This reduction in Cd availability leads to minimized Cd uptake by sunflowers, consequently lowering Cd-induced stress and oxidative damage. Furthermore, PGPR establishes a beneficial symbiosis with sunflower roots, promoting nutrient uptake, enhancing tolerance to water stress, and stimulating the production of stress-related compounds like proline. The positive impact of biochar and PGPR on plant productivity is evident in the significant improvements in sunflower growth, biomass, and yield parameters. Enhanced chlorophyll content and more efficient photosynthetic rates further support improved photosynthetic efficiency and carbon assimilation. Despite these advantages, certain limitations and future directions need to be considered. Further research should focus on determining the optimal application rates and combinations of biochar and PGPR for various soil types and sunflower cultivars. Long-term studies are essential to evaluate the sustained effects of these treatments on soil properties and plant performance. Additionally, scaling up these strategies to larger agricultural settings and addressing their economic feasibility are important factors for broader application.

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CRediT authorship contribution statement

Tarek Alshaal: Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation, Conceptualization. **Khadiga Alharbi:** Visualization, Funding acquisition. **Eman Naif:** Methodology, Investigation. **Emadelden Rashwan:** Visualization, Validation, Data curation. **Alaa El-Dein Omara:** Writing – original draft, Software, Conceptualization. **Emad M. Hafez:** Writing – original draft, Validation, Software, Resources, Formal analysis.

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.

Data Availability

Data will be made available on request.

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