www.nature.com/scientificreports

scientific reports

Check for updates

OPEN

Combine effects of *Broussonetia papyrifera*-derived biochar and selenium nanoparticles for lead-polluted saline soils remediation during barley cultivation

Ghulam Murtaza[1,2], Najmaldin Ezaldin Hassan[3], Muhammad Usman[4], Qamar Uz Zaman[1,5], Muhammad Rizwan[6], Gang Deng[1]✉, Zeeshan Ahmed[7]✉, Asma Majeed[8], Javed Iqbal[9], Mohamed S. Elshikh[10], Noorah AlKubaisi[10], Shabir Ahmad[11], Lala Gurbanova[12], Rashid Iqbal[12,13]✉, Urszula Malaga-Tobola[14] & Marek Gancarz[14,15,16]✉

Contamination of soil with lead (Pb) and salinity is a substantial concern, impacting plant health and yield. This research analyzes the synergistic use of *Broussonetia papyrifera*-derived biochar (BP-BC) and selenium nanoparticles (SeNPs) to alleviate the detrimental impacts of salinity and Pb on barley plants. Field trials in Pb-polluted saline soils showed the use of SeNPs (0, 10 and 20 mg L^{-1}) and BP-BC (0, 5 and 10 t ha^{-1}) markedly enhanced soil physicochemical attributes, such as soil ESP, pH and EC. The synergistic use of SeNPs and BP-BC substantially alleviated influences of salinity and Pb on barley plant and soil. BP-BC (10t-ha^{-1}) diminished pH of soil by 5.98% and exchangeable sodium percentage (ESP) by 29.71% relative to the control group, while enhancing microbial biomass (49.11%). SeNPs (20 mg L^{-1}) diminished Pb buildup in barley shoots by 60.39% and in seed by 49.40%, whereas combination of SeNPs and BP-BC resulted in reductions of 73.88% and 59.89%, respectively. These amendments increased activity antioxidant enzyme, with APX by 55.21% and (CAT) rising by 36.30%. The yield of barley grain rose by 39.11% with sole BP-BC application and by 80.20% with combination treatment of SeNPs and BP-BC, indicating their efficacy as environmentally sustainable soil supplements for Pb-polluted and saline soils. These findings highlight efficacy of SeNPs and BP-BC as environmentally benign additives for enhancing barley yield in polluted soils, presenting a viable approach for the sustainable agriculture in saline regions.

[1]School of Agriculture, Yunnan University, Kunming 650504, Yunnan, China. [2]School of Ecology and Environmental Sciences, Biocontrol Engineering Research Center of Crop Diseases & Pests, Yunnan University, Kunming 650500, Yunnan Province, China. [3]College of Engineering, Civil and Environment Department, University of Zakho, Kurdistan, Iraq. [4]School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China. [5]Department of Environmental Sciences, The University of Lahore, Lahore 54590, Pakistan. [6]Key Laboratory of Water-Sediment Sciences and Water Disaster Prevention of Hunan Province, School of Hydraulic and Environmental Engineering, Changsha University of Science & Technology, Changsha 410114, China. [7]Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, Xinjiang, China. [8]Institute of Agro-industry and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan. [9]Department of Botany, Bacha Khan University, Charsadda 24420, Khyber Pakhtunkhwa, Pakistan. [10]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia. [11]Department of Plant Sciences, Quaid-i-Azam University Islamabad, Islamabad 45320, Pakistan. [12]Department of Life Sciences, Western Caspian University, Baku, Azerbaijan. [13]Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan. [14]Faculty of Production and Power Engineering, University of Agriculture in Krakow, Balicka 116B, Krakow 30-149, Poland. [15]Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, Lublin 20-290, Poland. [16]Center for Innovation and Research on Pro-Healthy and Safe Food, University of Agriculture in Kraków, Balicka 104, Kraków 30-149, Poland. ✉email: denggang1986@ynu.edu.cn; zeeshanagronomist@yahoo.com; rashid.iqbal@iub.edu.pk; marek.gancarz@urk.edu.pl

Keywords Saline soil, Lead pollution, Se nanoparticles, Biochar, Barley, Biochemical

Up to 17% of the world's cropland, or approximately 242 million hectares, is contaminated with toxic heavy metals like lead, arsenic, and cadmium. This pollution affects around 1.4 billion people living in high-risk areas, posing serious threats to food security, public health, and ecosystems[1]. Lead not only degrades soil quality and negatively impacts plant growth but also poses substantial health hazards to animals and humans, as it permeates the food system and accumulates in various plant tissues[2]. In Pakistan, soils poisoned with lead are widespread in southern regions, where agricultural fields are irrigated with water from the Sadiqia canal, which is heavily polluted with metals including cadmium, lead, and nickel[3]. Analysis of soil and water samples reveals that lead is the primary pollutant, with the Sadiqia canal functioning as the principal irrigation source for the region's burgeoning agricultural sectors[4]. Treatment solutions for irrigation water are crucial, although frequently exceed the capabilities of numerous countries. Globally, around 70% of water withdrawals go towards agriculture, highlighting the need for sustainable water management practices. Utilizing wastewater for crops can be beneficial, but it requires careful management to mitigate risks. Studies suggest that treated wastewater can reduce the need for synthetic fertilizers by providing essential nutrients to crops. Implementing efficient irrigation systems like drip irrigation can minimize water waste by up to 60% compared to traditional surface irrigation methods. Moreover, adopting conservation agriculture practices, such as reducing tillage and using cover crops, can enhance soil water retention and reduce erosion by up to 20%. By combining these approaches, farmers can promote sustainable agriculture, conserve water resources, and maintain soil health[5–7].

In Pakistan, barley is a significant crop, with an average annual production of around 200,000 tons. It accounts for approximately 1.3% of the country's total cereal production. Barley is primarily used as a food source, with around 70% utilized for human consumption, including the production of sweets, bread, and pastries. The remaining 30% is used as animal feed, with barley straw being a valuable byproduct, providing essential nutrients for livestock. Additionally, Pakistan's barley crop yields an average of 2.5 tons per hectare, highlighting the crop's importance in the country's agricultural sector and food industry[8]. Barley is the primary cultivated crop in Pakistan, and the government is actively working to increase its cultivation to address the gap between demand and production[9]. Barley farming encounters considerable obstacles owing to inadequate soil health resulting from saline and declining irrigation water status, chiefly attributed to toxic metals poisoning, particularly Pb[10]. Consequently, researchers are intensifying their efforts to identify sustainable, eco-friendly alternatives sourced from organic materials to alleviate hazards associated with lead contamination in irrigation water and soil, aiming to enhance plant quality and soil health[11].

In this context, innovative solutions such as biochar and nanoparticles offer promising methods to mitigate the effects of abiotic stress. The promise of biochar with nanoparticles is compelling, offering significant benefits for improving plant resilience. A thorough analysis of the practical challenges, including cost, scalability, and environmental impact, is essential[12,13]. A contemporary strategy for alleviating the detrimental impacts of soil salinity and Pb pollution involves application of soil supplements[14]. Previous research has examined several amendments and biochar generated from different feedstocks showed notable efficacy as pollution barrier. It markedly enhances soil health, characteristics, and microbe's functions, thereby diminishing uptake of Pb and sodium and their build-up within aerial parts of plant[15]. *Broussonetia papyrifera* is a biological material consisting of cellulose, hemicellulose, lignin, extractives, silica and OM[9]. The rationale behind selecting *Broussonetia papyrifera* biomass waste for biochar production lies in its abundance, sustainability, and potential to valorize waste biomass. As a fast-growing plant species, *Broussonetia papyrifera* can be readily cultivated and harvested, providing a consistent feedstock for biochar production. Utilizing its biomass waste reduces waste disposal issues and environmental pollution while creating a valuable product. The resulting biochar can exhibit unique physicochemical properties, such as high surface area and nutrient content, making it an effective soil amendment and pollutant adsorbent, ultimately contributing to sustainable agriculture and environmental remediation practices. Biochar possesses low ash concentration of 4%, granting it a comparative advantage over alternative plant waste utilized for biochar production[16]. Moreover, biochar derived from plants waste have been shown to improve soil's OM and nutrients accessibility, especially in the saline-nature soils[10].

Biochar markedly improves plant resilience to saline and drought conditions by favourably affecting the soil's mechanical, chemical, and biological balance[17,18]. From a physical standpoint, biochar improves soil structure by increasing porosity, thereby enhancing water retention and aeration, which is especially crucial in arid environments[19]. This suggests that plants thrive with a stable water supply, alleviating the negative effects of inconsistent water availability. Biochar improves soil health by reducing salt toxicity through the adsorption of Na + ions and the release of essential cations like calcium and potassium[20,21]. This improves nutrient availability and elevates the soil's cation exchange capacity, hence facilitating plant growth[22,23]. Biochar biologically enhances microbial activity and fosters favourable symbioses, particularly with NPs, which can further improve plant stress tolerance[24,25]. Concurrently, NPs, through their capacity to improve nutrition and water absorption and augment plant resilience to adverse conditions, provide an alternative biological handle[26,27]. NPs significantly enhance plant resilience to environmental stressors, including salinity and drought, due to their distinctive capacity to form symbiotic relationships with plant roots[28,29]. Under salinity, NPs mitigate sodium ion toxicity and enhance potassium ion absorption, which is crucial for osmotic equilibrium and the preservation of ionic homeostasis.

NPs mitigates salt toxicity by modulating sodium ion transport and enhancing potassium absorption, hence maintaining growth, water efficiency, and metabolic function under combined stress[30]. Recent study has demonstrated that biochar alone is insufficient to completely prevent the translocation of Na or Pb in wheat species or to support plant performance without the integration of organic components that augment biochar's fundamental functions[31]. In this scenario, nanotechnology is recognized as a viable method to alleviate abiotic stressors, including soil salinity and heavy metal pollution, while enhancing agricultural output[32]. Nanoparticles, measuring less than 100 nm, exhibit unique physical and chemical properties, including an enhanced surface area, higher reactivity, and improved magnetic features compared to traditional plant growth regulators. These characteristics enable the rapid uptake of nanoparticles by plant stomata upon application, leading to minimal loss[33]. NPs are regarded as harmless, environmentally benign, and non-toxic at low concentrations, and they can be obtained via physiochemical processes[34]. In alkaline soils, Se availability to plants diminishes, necessitating application as a foliar spray for adequate absorption[35]. Although Se is more accessible in acidic-nature soils, it is low available in the alkaline-nature soils, necessitating the application of Se directly onto plant leaves for optimal uptake[36]. Exogenous Se, whether combined with citric acid, sugar alcohols, amino acids, for foliar application, exhibits low solubility in water[37].

Selenium nanoparticles (SeNPs) are among most prevalent nanocomposites utilized in agriculture, exhibiting considerable effects on enhancing plant development and resilience to abiotic challenges, such as soil heavy metal contamination and salinity. SeNPs exhibit enhanced efficacy relative to conventional Se forms[38]. Recent research has demonstrated that SeNPs can improve absorption of vital nutrients while inhibiting detrimental elements, such as toxic metals and sodium ions, owing to their accessibility. Further studies are necessary to explore potential of SeNPs in alleviating the combined loss from Pb pollution and salinity in soil in barley plants.

This research was done due to lack of published material on use of *Broussonetia papyrifera*-derived biochar and selenium nanoparticles (SeNPs) to mitigate the effects of lead (Pb) pollution and salinity on barley plants. This study aims to investigate soil-plant response to saline soil contaminated with lead, employing biochar generated from *Broussonetia papyrifera* and selenium nanoparticles as an effective remediation method. *Broussonetia papyrifera* biomass waste was likely selected for biochar production due to its high carbon content, abundance, and renewability. This plant species is known for its rapid growth rate, allowing for sustainable harvesting and processing into biochar. Utilizing waste biomass from *Broussonetia papyrifera* also provides an opportunity for waste valorization, reducing environmental pollution and disposal issues. The produced biochar can possess beneficial properties, such as high surface area, porosity, and nutrient content, making it suitable for soil amendment, pollutant adsorption and C sequestration applications, ultimately contributing to sustainable agriculture and environmental management practices. The emphasis is on their function in alleviating contamination impacts to enhance food safety and soil quality. The study will assess integrated treatment applied to both foliar and soil to (a) examine the bioaccumulation coefficient, translocation factor and bioaccumulation factor of lead from contaminated soil to various tissues of barley plants, (b) evaluate effects on soil physicochemical properties, enzymatic and microbial activity, (c) analyse biochemical and physiological alterations in barley, alongside expression levels of catalase, ascorbate peroxidase and manganese superoxide dismutase genes and concerning yield.

Materials and methods

Experimental design and description

The study was performed in Agricultural Research Centre Bahawalpur (29.3981° N, 71.6908° E), Pakistan, from October 2023 to March 2024, barley growing season exhibited an average high temperature of 35 °C and low of 20 °C in October, alongside rainfall of 143 mm and relative humidity of 43.08%. As of March 2024, average high temperature was 30 °C, lowest was 16 °C, rainfall was 89 mm and relative humidity rose to 56%. Soil samples were obtained for initial physiochemical analyses (Table 1).

Before the trial, water samples taken from Sadiqia canal, the main irrigation site, were studied. Water samples pH (7.24) and electrical conductivity (0.51 dSm^{-1}). The levels of SO_4, NH_4, Cl and Na were 0.09 mg, 1.62, 1.62, 3.97 and 198 mg L^{-1}, respectively. Ni; 0.776 mg L^{-1}, Arsenic; 0.026 mg L^{-1} Cr; 0.030 mg L^{-1}, Al; 0.021 mg L^{-1}, Cd; 0.35 mg L^{-1} and Hg; 0.021 mg L^{-1}. Pb concentration was 0.121 mg L^{-1}, beyond permissible irrigation threshold of 0.01 mg L^{-1}. Prior to trial, soil tilled twice to aerate surface layer and guarantee consistent treatment application. Additions of NPK fertilizers were applied by guidelines provided by PARC, Pakistan. The study area was segmented into plots of 2 m ×2.5 m, with treatments organized in completely randomized block design. Nine various treatments were implemented for every replication: control (unamended), BP-BC 5 t ha^{-1}, BP-BC 10 t ha^{-1}, SeNPs 10 mg L^{-1}, SeNPs 20 mg L^{-1}, BP-BC 5 t ha^{-1} + SeNPs 10 mg L^{-1}, BP-BC 5 t ha^{-1}+SeNPs 20 mg L^{-1}, BP-BC 10 t ha^{-1}+SeNPs 10 mg L^{-1} and BP-BC 10 t ha^{-1}+ SeNPs 20 mg L^{-1}. Barley seeds (*Hordeum vulgare* JAU-83) taken from RARI (Regional Agricultural Research Institute Bahawalpur) before starting the research, seeded at dose of 100 kg t ha^{-1}, in rows spaced 20 cm. Irrigation occurred 5 times at monthly intervals, from seedling to maturity, utilizing water from Sadiqia canal. Conventional agricultural methods were employed to facilitate optimal plant growth during barley's development. Permissions or licenses were obtained to collect Barley seeds (*Hordeum vulgare*, JAU-83) taken from (Regional Agricultural Research Institute Bahawalpur) and waste of Broussonetia papyrifera from from Lal Suhanra Park before starting the research. Dr. Rashid Iqbal, undertook the formal identification of the plant material (*Hordeum vulgare*, *Broussonetia papyrifera*) used in this study and deposited the specimen of *Broussonetia papyrifera* in the herbarium of university with deposition number (SAS-554) for future use.

Production of *Broussonetia papyrifera* Biochar and analysis (BP-BC)

Waste from stems and leaves of *Broussonetia papyrifera* was obtained from Lal Suhanra Park. After air-drying at ambient temperature, it was pulverized into smaller fragments, approximately 3 mm. The ground *Broussonetia papyrifera* waste underwent gradual pyrolysis at 450 °C for 4 h to generate biochar, obtained biochar kept in packets until its application ass oil treatment at dose of 10 tha^{-1}, introduced after second ploughing and prior to third. Before application of BP-BC in field trial, samples were tested to ascertain their characteristics as soil supplement, as detailed by Alessandrino[39]. The analysis indicated that biochar is abundant in vital minerals, comprising N (4.99 g kg^{-1}), K (202.40 g kg^{-1}), P (5.99 g kg^{-1}), Zn (89.8 g kg^{-1}), Mg (8.11 g kg^{-1}), and Ca (329.58 g

Property	Value
EC (dSm^{-1})	4.50 ± 0.04
pH	8.51 ± 0.02
CEC ($cmol_c kg^{-1}$)	4.89
Exchangeable Na (%)	17.01 ± 0.39
BD (gcm^{-3})	1.53
SOM ($g\ kg^{-1}$)	11.10 ± 0.19
OC%	1.18
OM %	0.81
SO_4^{2-} ($meq\ L^{-1}$)	17.10 ± 1.10
Cl^- ($meq\ L^{-1}$)	26.01 ± 1.09
HCO_3^-($meq\ L^{-1}$)	5.70 ± 0.06
K^+($meq\ L^{-1}$)	0.40 ± 0.02
Na^+($meq\ L^{-1}$)	26.9 ± 1.19
Mg^{2+}($meq\ L^{-1}$)	5.80 ± 0.10
Ca^{2+}($meq\ L^{-1}$)	6.90 ± 0.09
Avail. P ($mg\ kg^{-1}$)	2.79
Avail. K ($mg\ kg^{-1}$)	10.29
Total N %	0.20
Total P %	0.006
Total K %	0.04
Available N ($mg\ kg^{-1}$)	9.91 ± 0.10
Available K ($mg\ kg^{-1}$)	370 ± 19
Available N ($mg\ kg^{-1}$)	7.98 ± 010
Total lead	6.20 ± 0.11
Extractable lead	0.89 ± 0.02
Total Cd ($mg\ kg^{-1}$)	2.84
Available Cd ($mg\ kg^{-1}$)	0.42
Total Zn ($mg\ kg^{-1}$)	39.42
Available Zn ($mg\ kg^{-1}$)	5.37
Total Mn ($mg\ kg^{-1}$)	69.21
Available Mn ($mg\ kg^{-1}$)	9.78
Total Ni ($mg\ kg^{-1}$)	5.42
Available Ni ($mg\ kg^{-1}$)	0.63
Texture	Clayey

Table 1. Experimental soil's physiochemical qualities.

kg^{-1}). OM content was 27.60%, with C at 530 g kg^{-1}, CEC at 5.01 $cmol_c kg^{-1}$ and SSA of 17.38 m^2g^{-1}. Biochar exhibited EC of 1.24 dS m^{-1}, pH of 8.9, and ash content of 20.2% and Pb concentration of 0.02 mg kg^{-1}.

Production and characteristics of senps

This work utilized SeNPs with 50 nm particle size and purity of 99.9%, procured from Shanghai Rui Chu Bio-Tech, China, and manufactured via a chemical process. The procedure entailed dissolving Na_2SeO_3 (0.5 moles) and NaOH (1 mol) in individual flasks and agitating them for 20 min at 65 °C. The heated Na_2SeO_3 solution subsequently mixed dropwise into hot NaOH, and mixture maintained at 65 °C for 100 min. Resultant SeNPs were meticulously rinsed with sterile DI-water and homogenized at 65 °C utilizing ultrasonic vibrations[40]. The SeNPs were administered to plants via spraying 3 times at fifteen-day intervals, utilizing dosages of 0, 10, and 20 mg L^{-1}, and commencing 30-days post-planting. Uniform spraying conducted on barley plants, utilizing DI water for control application.

Soil analysis

Soil specimen was taken with a digging tool at depth of 0 to 20 cm to assess physiochemical parameters of soil during harvest. The pH of soil was assessed following soil and foliar applications in growth phase through creating a suspension (water-soil at 1:2.25 ratios). Soil paste extract made from separate specimen to ascertain EC employing a pH meter (Lutron PH-223) and an EC meter (EC700), respectively[41]. The Exchangeable Na percentage (ESP) was determined utilizing this equation:

$$ESP = 1.95 + 1.03xSAR\ \left(R^2 = 0.92\right) \quad (1)$$

Whereas SAR (Sodium Adsorption Ratio)

$$SAR = [\text{Na+}]\frac{\sqrt{([\text{Ca2+}] + [\text{Mg2+}])}}{2} \tag{2}$$

Where Mg^{2+}, Ca^{2+}, and Na^{+} quantified in meq L^{-1}[42].

Biological activity soil was evaluated 85 days post-sowing by obtaining soil samples to quantify CO_2 efflux and microbial biomass C, adhering to methodologies described by[43]specifically utilizing fumigation incubation–extraction technique. Quantifying microbial biomass C in the soil is crucial, as it functions as a sign of microbial community's size and its function in OM dynamics and nutrient cycling[44]. Furthermore, the activities of soil enzymes were assessed 85 days post-sowing by collecting soil samples to quantify alkaline phosphatase and dehydrogenase activities. Dehydrogenase function in soil is crucial since it acts as a primary indication of microbial oxidative activities and total soil biological health. The activity of alkaline phosphatase in the soil is significant as it indicates soil's ability to mineralize organic P into accessible forms for crop uptake[45]. The activity of dehydrogenase was quantified utilizing spectrophotometer (YR06035) according to [68] employing 2, 3, 5-TTC technique (3% w/v), while activity of alkaline phosphatase was assessed with 4-nitrophenyl phosphate as a substrate, with absorbance recorded at 440 nm, as outlined by George et al.[46]. The bacteriological activities were assessed by quantifying the total bacterial count, Bacillus, and Azotobacter in soil samples at 75 days, utilizing King's B agar medium, according to methodologies outlined by Kizilkaya[47]. Assessing Bacillus and Azotobacter in the soil is crucial, as they serve as vital signs of microbial health and facilitate nitrogen fixation, nutrient cycling, and the promotion of plant growth[48]. During the harvest, soil samples were obtained to assess Pb concentration, extracted with EDTA, and measured via AAS (AAS 700, Perkin Elmer).

Pb translocation, intake as well as accumulation

Upon reaching physiological maturity, 5 barley plants randomly chosen from every trial plot, extracted, and meticulously cleaned. After crushing, samples were preserved in individual plastic bags. Materials were subsequently treated via Kjeldahl digestion procedure and incubated in an oven at 140 °C for 65 min, after this creation, Pb level in different tissues of barley plant was quantified utilizing AAS (AAS 700, Perkin Elmer). Pb translocation, uptake as well as accumulation were computed with these Eq[49].:

$$BCF = Pb\, in\, roots/Pb\, in\, soil \tag{3}$$

$$TF = Pb\, in\, leaves/Pb\, in\, roots \tag{4}$$

$$BAC = Pb\, in\, leaves/Pb\, in\, soil \tag{5}$$

Where BAC, TF, and BCF represent bioaccumulation factor, transfer factor, and bioconcentration factor, respectively.

Uptake of nutrient in leaves

Following 75-days of growing, leaves systematically gathered from each trial plot and preserved in containers for air-drying procedure. Leaves were desiccated in the oven at 75 °C and subsequently pulverized into fine powder. Powdered specimen was subjected to digestion with 1:1 combination of $HClO_4$ and HNO_3 for 100 min at 200 °C to quantify K concentration utilizing flame photometer. The levels of Na, Mg and Zn were evaluated utilizing AAS (AAS 700, Perkin Elmer).

Assessment of antioxidant and oxidative stress

To quantify catalase level, at first, phosphate buffer (5 mL) was mixed with 0.2 g of finely crushed leaf sample. Subsequently, solution was subjected to centrifugation at speed of 1500 rpm for the 20 min, after which the supernatant was separated perpendicularly. Afterward, a cuvette was filled with 0.1 mL of sample, H_2O_2 (1 mL), and phosphate buffer (1.9 mL). The absorbance at 240 nm was determined using spectrophotometer at time intervals of 0, 30, 60, and 90 s, as the level of ascorbate peroxidase was measured with technique stated through[50]. The reaction mixture used to determine APX consisted of 200 µl of a 2 mM ascorbate solution mixed in a 100 mM phosphate buffer with 7 pH, 200 µL of a 10 mM hydrogen peroxide solution, 30 µL of a 5 mM EDTA solution, and protein extract (20 µL). The mixed reaction's absorbance was determined at wavelength of 290 nm, and APX level was measured with an extinction coefficient of 2.8 $mM^{-1}cm^{-1}$. To test concentration of H_2O_2 (Hydrogen peroxide), a solution of phosphate buffer (50 mM, pH 6.6) was mixed with 50 mg leaf samples to create uniform mixture. The mixture was then subjected to centrifugation at 6000×g for 30 min at a temperature of 4 °C. To test the concentration of H_2O_2, solution of phosphate buffer (H_2KO_4P) (50 mM, pH 6.5) was mixed with 50 mg leaf samples to create uniform mixture. The mixture was then subjected to centrifugation at 6000×g for 30 min at temperature of 4 °C. Next, 1 mL of a solution containing 0.1% titanium sulfate in 20% (v/v) sulfuric acid was added to extracted solution. The resulting combination was then subjected to centrifugation at a force of 6000 ×g for 20 min. The centrifugation was carried out at regulated temperature of 4 °C. Supernatant's absorbance was quantified at wavelength of 410 nm. The H_2O_2 concentrations were computed with extinction value of 0.28 $\mu mol^{-1}cm^{-1}$., as described by Zawoznik et al.[51]. Lipid peroxidation was measured as MDA (malondialdehyde) at 450 nm, 532 nm, and 600 nm with spectrophotometer, in accordance with the method outlined by Alharbi et al.[34]. EL levels in the shoots were measured through removing one plant from each replication and treatment. Samples were separated into small pieces and positioned vertically in tubes containing 7 mL of DI water. Initial electrical conductivity of solution was measured after incubating tubes at temperature of 32 °C for 2 h. Samples were subjected to autoclaving for duration of 20 min at a temperature of 120 °C. The final electrical conductivity of the solution was measured after sample was cooled to 25 °C. The EL was computed with equation provided by Eq. 6, as specified by Alshaal et al.[52]

$$EL = (EC1 - EC2) \times 100 \tag{6}$$

Physiological activity of plants

75 days post-planting, SPAD leaf greenness indices were assessed utilizing chlorophyll meter (SPAD-502Plus), as described by Szulc et al.[53]. The stomatal conductivity (Gs) and rate of photosynthesis (Pn) were measured with open infrared gas analyzer (LI-6800), according to methodology outlined by Xie and Su[54]. The proline level was quantified through pulverising 0.25 g of freshly harvested leaf material in 5 mL of a 3% $C_7H_6O_6S$ solution, and subsequently filtering the resulting extract. A volume of 1 mL of the filtrate was kept in a test tube together with 1 mL of $C_6H_4(CO)_2C(OH)_2$ and 1 mL of CH_3COOH. Mixture was then heated in water bath for 90 min at temperature of 100 °C. Two distinct layers were formed as a result of the vortexing process. Absorbance of the upper layer, which had a pinkish colour, was determined at wavelength of 520 nm with spectrophotometer, as described by Filek et al.[55].

Transcriptional analysis of antioxidants

The qPCR research was conducted to assess the expression genes in antioxidants (Mn-SOD, APX, and CAT) in barley plants exposed to saline-nature soil watered with wastewater. 3 replicates were chosen for extraction of cDNA and RNA utilizing Qiagen kits. The PCR procedures and amplification protocols for Mn-SOD, APX, and CAT genes conducted as outlined by Kumar et al.[56]. The expression values of antioxidant genes quantified utilizing $2^{-\Delta\Delta Ct}$ technique, with ACTIN as internal reference gene[57].

Yield assessment

During the harvest period, 5 plants from every plot chosen to evaluate yield-characteristics, such as grain count per spike and 1000 grains weight (g). Furthermore, 2 m^2 sections from center of every plot were collected to assess grain yield at 14% moisture level.

Statistical study

The collected data were analyzed using analysis of variance (ANOVA) procedures, utilizing the CoStat 6.4 Statistical Software package. When significant differences were found ($p < 0.05$), mean comparisons were performed according to the method outlined by James et al.[58].

Results

Soil's physicochemical characteristics

Despite adverse impacts of Pb-polluted saline soils on soil physicochemical attributes (such as ESP, EC, and pH) and microbial traits (including microbial biomass and soil CO_2), soil amendments application such as BP-BC, SeNPs through foliar spray, and their combination-substantially enhanced these characteristics (Table 2). BP-BC alone showed superior efficacy than SeNPs in improving soil microbiological and physicochemical characteristics. Specifically, the application of BP-BC at 5 t ha^{-1} and 10 t ha^{-1} diminished pH (3.01–5.98%), ESP (20.29–29.71%), and EC (9.23–24.40%) in comparison to control group. BP-BC also enhanced microbial biomass (80.04-149.11%), and CO_2 influx (56.39-119.59%) at similar levels. Conversely, SeNPs foliar application (10 and 20 mg L^{-1}) resulted in marginal reduction of exchangeable sodium percentage (ESP) by 0.16%, while enhancing microbial biomass (7.39–5.49%) and carbon dioxide influx (7.11–5.60%) in comparison to control group.

Treatments	Dose	EC (dS m^{-1})	pH	ESP	CO_2 influx (mg CO_2 100 g^{-1} soil 24 h^{-1})	Microbial biomass (mg g^{-1} soil)
BP-BC (t ha^{-1})	0	4.50a ± 0.09	8.5a ± 0.4	17.19a ± 0.89	16.01c ± 1.19	2.30c ± 0.29
	5	4.1b ± 0.20	8.2b ± 0.19	14.30b ± 0.59	25.80b ± 1.30	4.10b ± 0.10
	10	3.40c ± 0.19	7.90c ± 0.39	12.10c ± 0.49	36.70a ± 1.40	5.59a ± 0.20
SeNPs (mg L^{-1})	0	4.1a ± 0.29	8.2a ± 0.19	14.60a ± 0.79	25.40b ± 1.79	3.80b ± 0.39
	10	4.1a ± 0.30	8.19a ± 0.38	14.60a ± 0.49	25.9a ± 1.19	3.99a ± 0.50
	20	4.2a ± 0.19	8.2a ± 0.29	14.61a ± 0.37	26.80a ± 1.70	4.2a ± 0.51
BP-BC + SeNPs	0	4.3a ± 0.30	8.3a ± 0.20	14.78a ± 0.80	26.43b ± 1.80	4.80b ± 0.40
	10	4.3a ± 0.29	8.10a ± 0.40	14.69a ± 0.50	26.91a ± 1.20	4.99a ± 0.51
	20	4.3a ± 0.20	8.3a ± 0.30	14.72a ± 0.40	27.82a ± 1.72	5.2a ± 0.52
p BP-BC		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
p SeNPs		0.28	0.26	< 0.01	< 0.01	< 0.01
p BP-BC + SeNPs		0.69	0.90	0.98	0.80	< 0.05

Table 2. SeNPs and BP-BC impacts on soil Microbiological and physicochemical characteristics in Pb-polluted saline soils. Values represent the mean ± SD of 3 replicates. Distinct letters denote statistically significant differences among treatments as per Tukey's HSD test (p irrigation × treatment < 0.05)

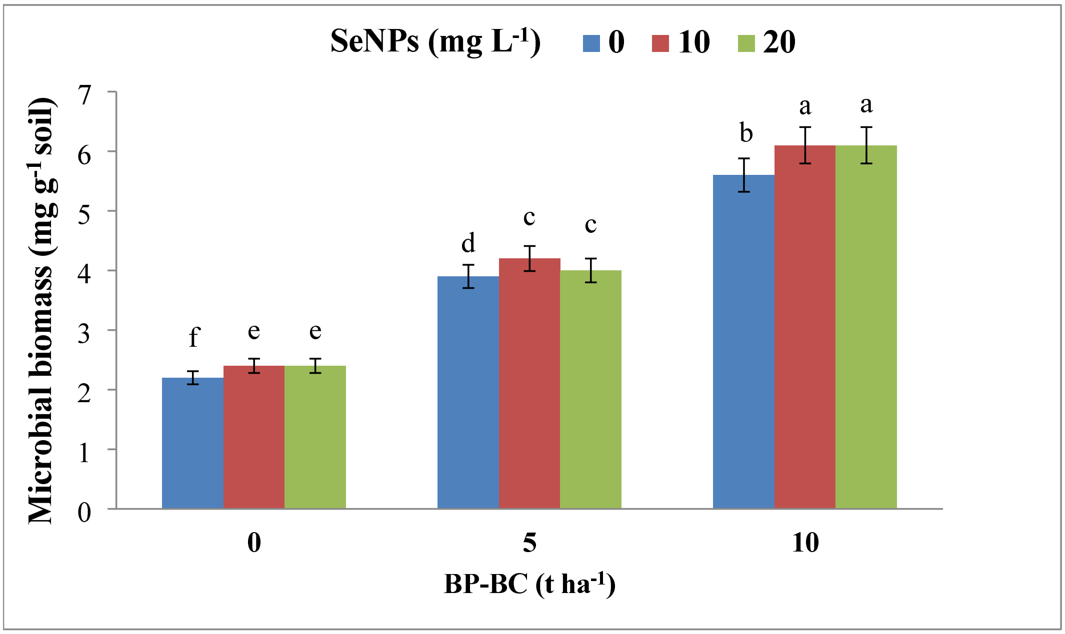

Fig. 1. Interaction effect of SeNPs and BP-BC on microbial biomass level. Distinct letters on bars indicate significant differences at the $p \le 0.05$ levels as per Tukey test. Data are expressed as mean ± standard deviation (SD).

Treatments	Dose	Alkaline phosphatase (mg phenol kg^{-1} day^{-1})	Dehydrogenase (mg phenol kg^{-1} day^{-1})	Bacteria (Log cfu g^{-1} soil)	Bacillus (Log cfu g^{-1} soil)	Azotobacter (Log cfu g^{-1} soil)
BP-BC (t ha^{-1})	0	0.30c ± 0.019	13.40c ± 0.39	2.70c ± 0.30	1.10c ± 0.30	0.96c ± 0.20
	5	0.49 ± 0.030	30.40b ± 1.10	5.1b ± 0.40	3.80b ± 0.20	2.30b ± 0.25
	10	0.80a ± 0.031	45.19a ± 0.60	7.80a ± 0.50	5.80a ± 0.19	4.50a ± 0.40
SeNPs (mg L^{-1})	0	0.6b ± 0.19	30.09b ± 0.09	4.80b ± 0.30	3.49b ± 0.69	2.19b ± 0.39
	10	0.60a ± 0.19	31.35a ± 0.89	5.50a ± 0.40	3.89a ± 0.70	2.69a ± 0.60
	20	0.49a ± 0.16	31.10a ± 0.79	5.39a ± 0.30	4.1a ± 0.72	2.80a ± 0.59
BP-BC + SeNPs	0	0.7b ± 0.19	31.09b ± 0.09	5.79b ± 0.30	4.99b ± 0.69	3.19b ± 0.39
	10	0.70a ± 0.19	32.35a ± 0.89	6.51a ± 0.40	4.90a ± 0.70	3.69a ± 0.60
	20	0.51a ± 0.16	32.10a ± 0.79	6.40a ± 0.30	5.2a ± 0.72	3.80a ± 0.59
p BP-BC		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
p SeNPs		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
p BP-BC + SeNPs		0.79	0.39	< 0.01	< 0.01	< 0.01

Table 3. SeNPs and BP-BC Imapcts on dehydrogenase, alkaline phosphatase activity, bacterial counts, Azotobacter populations and Bacillus populations in barley plants cultivated in Pb-polluted saline soils. Values represent the mean ± SD of 3 replicates. Distinct letters denote statistically significant differences among treatments as per Tukey's HSD test (p irrigation × treatment < 0.05)

Figure 1 illustrates that the combined use of BP-BC at a rate of 10 t ha^{-1} and SeNPs at 10 mg L^{-1} resulted in the most significant enhancement of microbial biomass, with a 170.59% increase. Interaction between SeNPs and BP-BC did not dramatically influence soil physicochemical parameters, except microbial biomass level, which exhibited clear-cut positive interaction.

Bacteriological activity and soil enzymes

Single-use of soil supplements, such as foliar sprays of SeNPs and BP-BC and their combination, markedly improved microbiological and soil enzyme activity. The findings in Table 3 demonstrate that sole use of BP-BC significantly enhanced soil enzyme activity, particularly alkaline phosphatase and dehydrogenase, as well as microbial activity of *Bacillus*, bacteria, and *Azotobacter* relative to SeNPs foliar application. In a comparison of treatments utilizing BP-BC to control group (BP-BC-without), BP-BC use at 5 t ha^{-1} and 10 t ha^{-1} led to substantial enhancements in several metrics: alkaline phosphatase by 85.70-169.80%, the activity of dehydrogenase increased by 139.33–260.20%, bacterial numbers by 98.10-189.39%, *Bacillus* by 98.30-210.70% and *Azotobacter* by 140.12–380.20%. A significant interaction was noted between the combined use of SeNPs

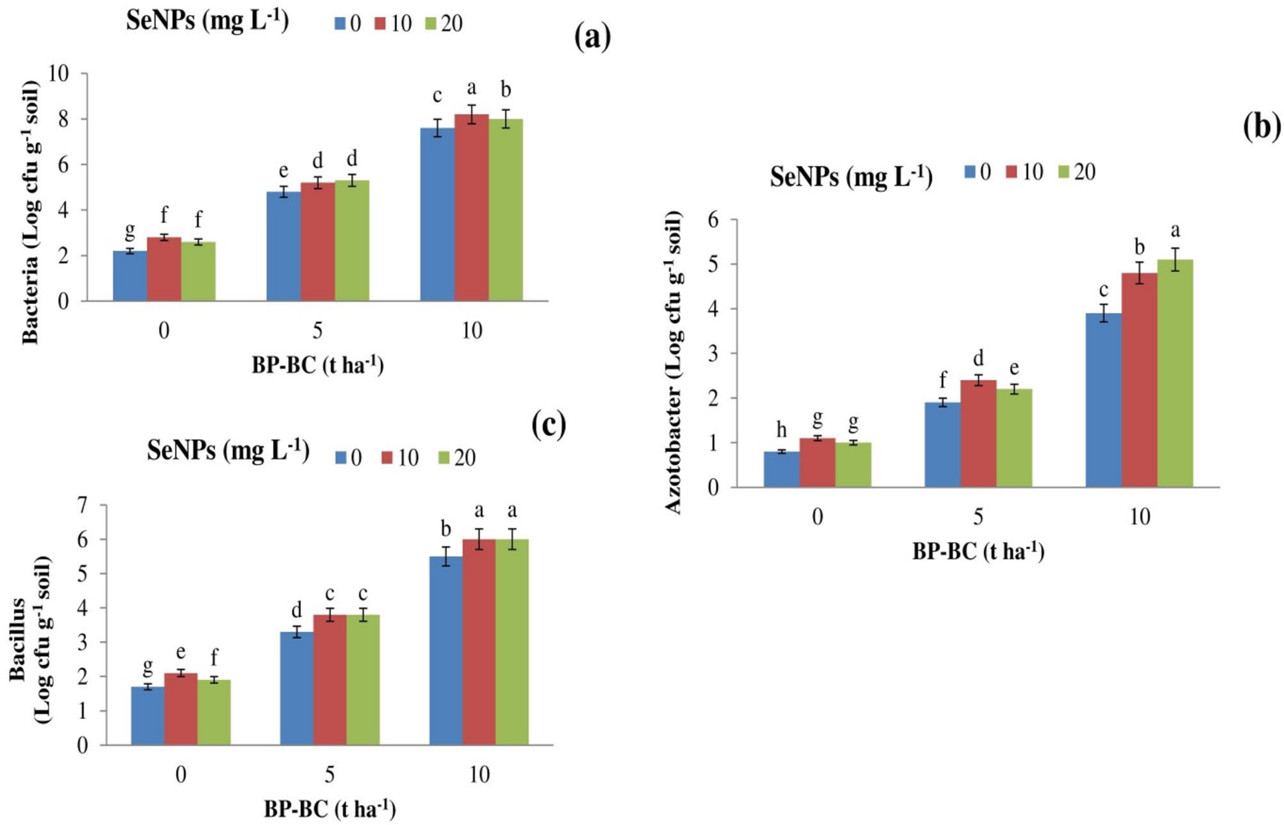

Fig. 2. Interaction effects of SeNPs and BP-BC on bacteria (**a**), *Azotobacter* (**b**), and *Bacillus* (**c**). Distinct letters on bars indicate significant differences at the $p \le 0.05$ levels as per Tukey test. Data are expressed as mean ± standard deviation (SD).

Treatments	Dose	Soil Pb (mg kg^{-1})	Roots Pb (µg g^{-1})	Shoots Pb (µg g^{-1})	Seeds Pb (µg g^{-1})
BP-BC (t ha^{-1})	0	0.90a ± 0.010	3.01a ± 0.14	1.60a ± 0.06	0.9a ± 0.02
	5	0.8b ± 0.019	1.90b ± 0.10	1.13b ± 0.10	0.60b ± 0.05
	10	0.60c ± 0.021	1.40c ± 0.20	0.70c ± 0.03	0.3c ± 0.01
SeNPs (mg L^{-1})	0	0.80a ± 0.060	2.30a ± 0.70	1.40a ± 0.10	0.69a ± 0.03
	10	0.8b ± 0.039	1.89b ± 0.69	0.98b ± 0.09	0.49c ± 0.05
	20	0.69b ± 0.040	1.88b ± 0.8	0.79c ± 0.06	0.55b ± 0.05
BP-BC + SeNPs	0	0.90a ± 0.060	3.30a ± 0.70	2.40a ± 0.10	0.79a ± 0.03
	10	0.9b ± 0.039	2.89b ± 0.69	1.10b ± 0.09	0.69c ± 0.05
	20	0.79b ± 0.040	2.88b ± 0.8	1.33c ± 0.06	1.10b ± 0.05
p BP-BC		< 0.01	< 0.01	< 0.01	< 0.01
p SeNPs		< 0.01	< 0.01	< 0.01	< 0.01
p BP-BC + SeNPs		0.16	0.13	< 0.01	< 0.01

Table 4. Impact of senps and BP-BC on extractable soil Pb concentration, Pb levels in roots, Pb levels in shoots, and Pb levels in seeds of barley plants cultivated in Pb-polluted saline soils.

(10 mg L^{-1}) and BP-BC (10 t ha^{-1}), resulting in enhancements in *Bacillus* (290.80%) and bacteria populations (260.30%) relative to control group. Moreover, as illustrated in Fig. 2, combined use of SeNPs (20 mg L^{-1}) and BP-BC (10 t ha^{-1}) produced a markedly higher enhancement in *Azotobacter* (560.70%) compared to control.

Accumulation of Pb in soil and various plant parts

The findings in Table 4 demonstrate that SeNPs and BP-BC application, whether separately or in conjunction, markedly diminished Pb buildup in soil, shoots, roots, and seeds. Significantly, BP-BC sole addition was more efficacious in reducing Pb concentrations in both plant tissues and soil than the SeNPs application. Specifically, application of BP-BC at 5 t ha^{-1} and 10 t ha^{-1} led to reductions in Pb accumulation in the following ways:

in the soil (19.37–35.67%), in shoots (34.01–60.39%), in roots (38.40–4.70%), and in seeds (28.29–49.40%), then control. Furthermore, the SeNPs foliar application at doses of 10 and 20 mg L^{-1} resulted in decreased Pb buildup in soil (3.70–3.40%), shoots (29.19–37.18%), roots (12.03–13.70%) and seeds (27.40–24.30%) than control (SeNPs 0 mg L^{-1}). Figure 3 demonstrates that mixed use of SeNPs and BP-BC did not exhibit significant interactions with Pb concentrations in the roots or soil; nevertheless, a notable interaction was seen in the seeds and shoots. The combined use of SeNPs (10 mg L^{-1}) and BP-BC (10 t ha^{-1}) significantly reduced Pb build-up in seeds, attaining a reduction of 59.89%. Furthermore, combined administration of SeNPs (20 mg L^{-1}) and BP-BC (10 t ha^{-1}) caused in substantial decrease in Pb build-up in plant shoots, particularly 73.88%, in contrast to the treatment with SeNPs (0 mg L^{-1}) and BP-BC (0 t ha^{-1}).

Pb translocation, accumulation, and uptake

Figure 4 demonstrates that the administration of SeNPs and BP-BC altered Pb accumulation in different tissues of barley plants relative to control, resulting in decreased BAC, TF, and BCF. The SeNPs foliar application significantly reduced TF more than the BP-BC treatment. The BP-BC application at 5 t ha^{-1} and 10 t ha^{-1} led to reductions in BAC (18.30–37.70%), TF (4.99–10.11%) and BCF (19.30–32.10%) compared to control (0 t ha^{-1} BP-BC). Likewise, SeNPs foliar application at doses of 10 and 20 mg L^{-1} resulted in decreases in BAC (28.08–35.40%), TF (19.98–27.48%) and BCF (10.30–11.60%) compared to control (SeNPs 0 mg L^{-1}) in Pb-polluted saline soils. Figure 4 demonstrates that the synergistic use of SeNPs and BP-BC significantly influenced BAC, TF, and BCF in barley plants cultivated in Pb-polluted salty soils (Fig. 4a-c). The combined use of BP-BC (10 t ha^{-1}) and SeNPs (20 mg L^{-1}), led to significant decreases in BAC (60.39%), TF (36.20%) and BCF (38.39%) relative to BP-BC (0 t ha^{-1}) and SeNPs (0 mg L^{-1}) treatments.

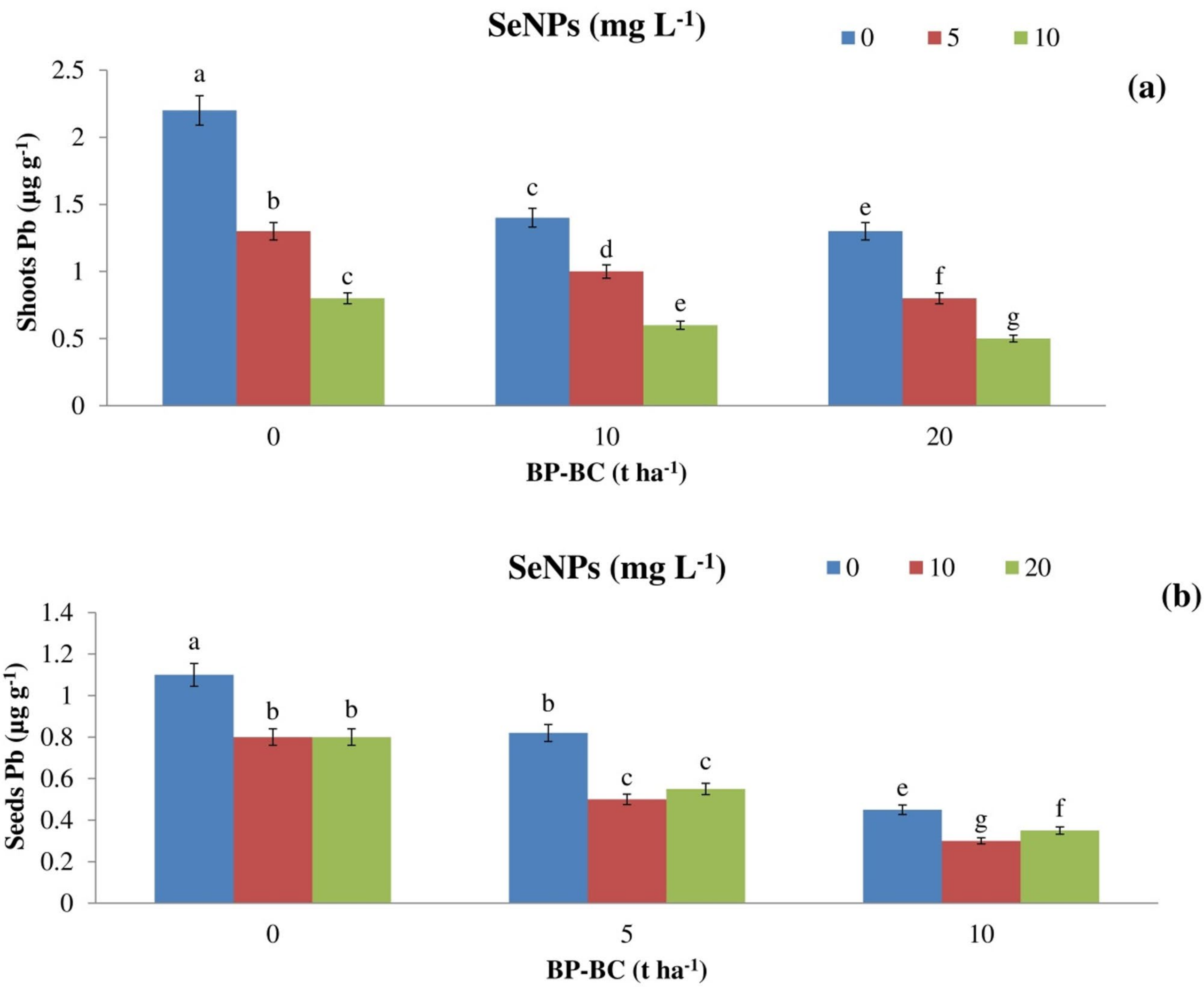

Fig. 3. Interactive effect of SeNPs and BP-BC on the shoot Pb (**a**) and seeds Pb (**b**). Distinct letters on bars indicate significant differences at the $p \leq 0.05$ levels as per Tukey test. Data are expressed as mean ± standard deviation (SD).

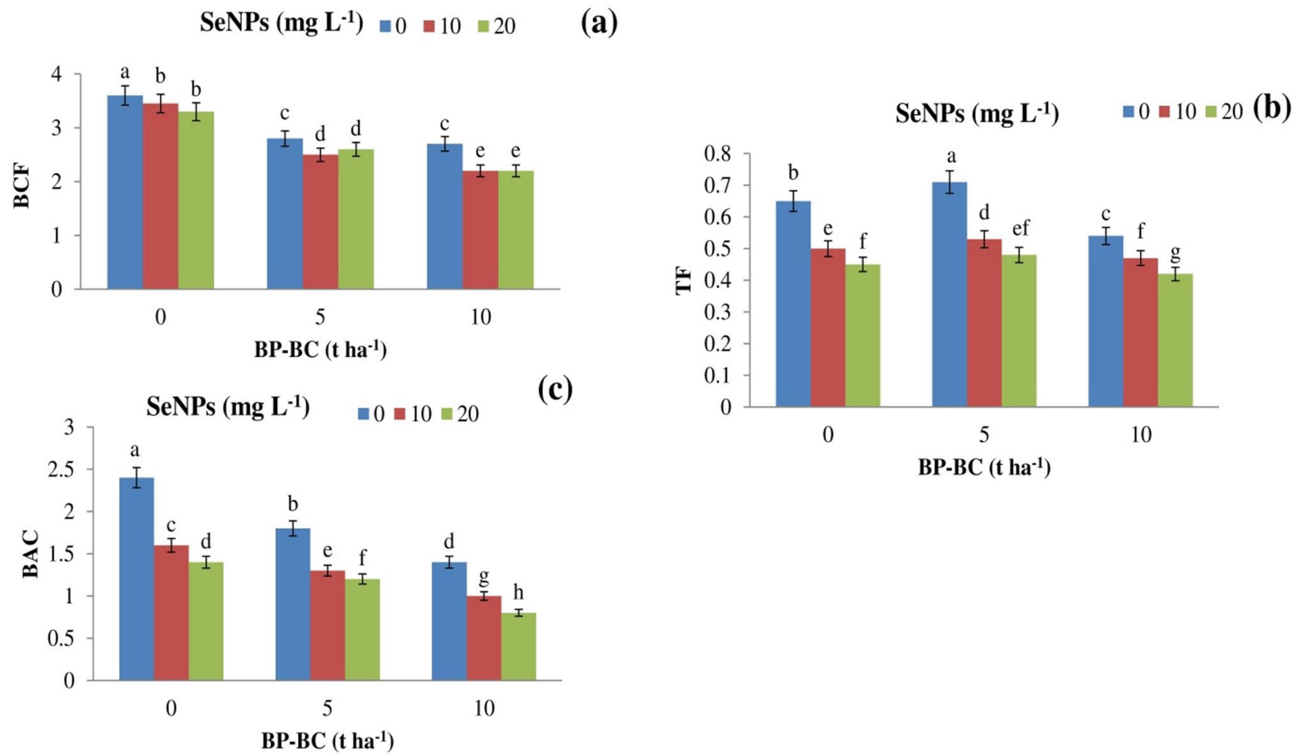

Fig. 4. Interaction effect of SeNPs and BP-BC on (**a**) BCF), (**b**) TF and (**c**) BAC, Distinct letters on bars indicate significant differences at the $p \leq 0.05$ levels as per Tukey test. Data are expressed as mean ± standard deviation (SD).

Leaves nutrient uptake

Despite detrimental effects of Pb-polluted saline soils on Zn, Mg, and K uptake in barley plants, SeNPs and BP-BC application can improve absorption of Zn, Mg, and K, even in such unfavorable conditions. The BP-BC alone administration markedly decreased Na uptake while concurrently increasing Zn, Mg, and K uptake in leaves than SeNPs sole use. The BP-BC addition (5 t ha^{-1} and 10 tha^{-1}) led to decrease in Na uptake (30.80-49.87%) and an increase in Zn uptake (47.83-100.95%), Mg (43.59–86.50%) and K (90.47-190.33%) compared to control (BP-BC t ha^{-1}). The SeNPs foliar addition at doses of 10 and 20 mg L^{-1} caused in decrease in sodium uptake (30.87–26.74%), while enhancing the potassium uptake (67.22–68.18%), magnesium (29.70-33.27%) and zinc (63.51–67.79%) compared to control treatment (SeNPs0 mg L^{-1}). Figure 5 similarly illustrates that concurrent use of SeNPs and BP-BC exhibited notable interaction regarding accumulation of Zn, Mg, K and Mg in barley plants cultivated in Pb-polluted saline-nature soils (Fig. 5a-d). The BP-BC use (10 t ha^{-1}) in conjunction with SeNPs (20 mg L^{-1}) caused significant decrease in build-up of Na (63.79%), while simultaneously increasing K accumulation (949.87%), Zn (240.30%) and Mg (139.70%) compared to control treatment (BP-BC 0 t ha^{-1} and SeNPs 0 mg L^{-1}).

Assessment of antioxidant and oxidative stress

Table 5 demonstrates that treatment of SeNPs and BP-BC and combined use markedly elevated APX and CAT amounts, while concurrently diminishing H_2O_2 and MDA concentrations in leaves subjected to Pb-polluted saline-nature soil. Data demonstrate that BP-BC sole produced more significant elevation in APX and CAT amounts, as well as a more pronounced decrease in H_2O_2 and MDA, in comparison to the alone use of SeNPs. The application of BP-BC (5 t ha^{-1} and 10 t ha^{-1}) elevated APX concentration by 30.39–55.21% and CAT amounts by 19.10–36.30%, while concurrently decreasing H_2O_2 by 29.33–56.80% and MDA by 27.28–56.14% in comparison to BP-BC (0 t ha^{-1}). Conversely, the SeNPs foliar application at rates of 10 and 20 mg L^{-1} enhanced catalase (CAT) activity by 16.74–18.28% and ascorbate peroxidase (APX) activity by 18.30-19.78%, while simultaneously decreasing malondialdehyde (MDA) levels by 28.40-31.44% and hydrogen peroxide (H_2O_2) levels by 29.15–33.50% in comparison to SeNPs (0 mg ha^{-1}).

The simultaneous SeNPs and BP-BC application did not produce a significant interaction concerning MDA or CAT levels; however, a distinct interaction was observed with H_2O_2 and APX levels. The simultaneous use of SeNPs (20 mg L^{-1}) and BP-BC (10 t ha^{-1}) led to a notable enhancement in APX level (86.79%) and a decrease in H_2O_2 level (69.11%) relative to control group.

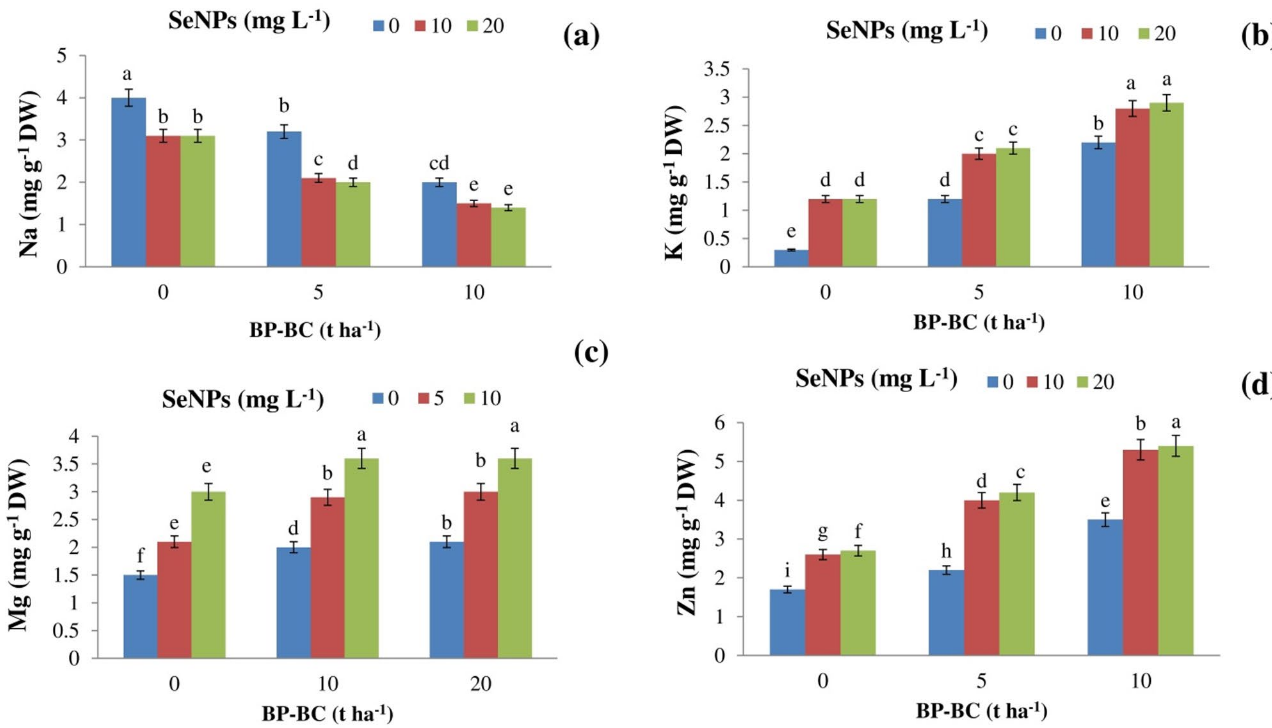

Fig. 5. Interaction effects of SeNPs and BP-BC on (**a**) Na, (**b**) K, (**c**) Mg and (**d**) Zn. Distinct letters on bars indicate significant differences at the $p \le 0.05$ levels as per Tukey test. Data are expressed as mean ± standard deviation (SD).

Treatments	Dose	APX (μmol H_2O_2 g^{-1}FW min^{-1})	CAT (μmol H_2O_2 g^{-1}FW min^{-1})	H_2O_2 ((μmol g^{-1} FW)	MDA ((μmol g^{-1} FW)
BP-BC (t ha^{-1})	0	12.99c ± 0.19	46.1c ± 1.60	7.70a ± 0.49	19.30a ± 0.59
	5	17.11b ± 0.80	54.50b ± 2.19	5.30b ± 0.29	14.50b ± 0.89
	10	21.19a ± 0.39	59.30a ± 2.40	3.40c ± 0.30	8.3c ± 0.69
SeNPs (mg L^{-1})	0	15.01c ± 0.88	50.10b ± 1.39	7.01a ± 0.19	17.50a ± 0.89
	10	18.11b ± 1.2	56.80a ± 2.15	4.79b ± 0.88	12.1b ± 0.50
	20	18.41a ± 1.10	57.40a ± 2.29	4.60c ± 0.59	12.44 ± 0.46
BP-BC + SeNPs	0	16.01c ± 0.88	51.10b ± 1.39	8.01a ± 0.19	18.50a ± 0.89
	10	19.11b ± 1.2	58.80a ± 2.15	5.79b ± 0.88	13.1b ± 0.50
	20	19.41a ± 1.10	58.40a ± 2.29	5.60c ± 0.59	13.44 ± 0.46
p BP-BC		< 0.01	< 0.01	< 0.01	< 0.01
p SeNPs		< 0.01	< 0.01	< 0.01	< 0.01
p BP-BC + SeNPs		< 0.01	0.55	< 0.01	0.16

Table 5. Impact of senps and BP-BC on CAT, APX, MDA, and H_2O_2 in barley plants subjected to Pb-polluted saline soils.

Physiological activity of plants

Pb-polluted saline soils negatively impact physiological functions in plants, resulting in disruptions in the membrane leaking, leaf greenness (assessed through SPAD), stomatal conductance, photosynthesis rate, as well as leaves proline level (Table 6). The SeNPs and BP-BC utilization, whether separately or in conjunction, significantly reduced proline concentrations and membrane leakage, while simultaneously improving leaf greenness, photosynthetic efficiency, and stomatal conductivity. Table 6 demonstrates that the alone BP-BC application was more efficacious in diminishing proline concentrations and membrane leakage, while also enhancing leaf greenness, stomatal conductivity and photosynthetic rate in comparison to sole administration of SeNPs. In the comparison of treatments, the BP-BC (5 t ha^{-1} and 10 tons ha^{-1}) application led to a decrease in proline level (33.4059.30%) and membrane leakage (19.30-44.59%), while enhancing leaf greenness (30.88–56.22%), stomatal conductivity (27.22–53.70%), and photosynthetic rate (37.71–80.49%) compared to control (BP-BC 0 t ha^{-1}). The SeNPs foliar application at dosages of 10 and 20 mg L^{-1} resulted in decreased proline

Treatments	Dose	Membrane leakage (%)	Pn (μmol $m^{-2}s^{-1}$)	Leaf greenness (SPAD)	Gs (mmol H_2O $m^{-2}s^{-1}$)	Proline level (mg 100 g^{-1} FW)
BP-BC (t ha^{-1})	0	37.91a ± 1.70	11.45c ± 0.50	30.21c ± 1.10	36.01c ± 1.59	10.99a ± 0.49
	5	30.29b ± 1.49	15.10b ± 0.89	37.60b ± 2.15	46.25b ± 1.89	7.50b ± 0.30
	10	22.20c ± 1.09	20.47a ± 1.13	44.81a ± 1.30	55.79a ± 2.40	4.40c ± 0.50
SeNPs (mg L^{-1})	0	34.10a ± 1.49	13.01b ± 0.90	32.8b ± 1.30	39.78b ± 2.87	9.50a ± 0.89
	10	28.10b ± 1.29	17.10a ± 0.78	40.24a ± 1.70	48.7a ± 1.99	6.10b ± 0.40
	20	27.89b ± 1.80	17.03a ± 1.10	40.89a ± 1.39	51.44a ± 2.82	6.50c ± 0.69
BP-BC + SeNPs	0	35.10a ± 1.49	14.01b ± 0.90	33.8b ± 1.30	40.78b ± 2.87	10.50a ± 0.89
	10	29.10b ± 1.29	18.10a ± 0.78	41.24a ± 1.70	49.7a ± 1.99	7.10b ± 0.40
	20	28.89b ± 1.80	18.03a ± 1.10	41.89a ± 1.39	52.44a ± 2.82	7.50c ± 0.69
p BP-BC		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
p SeNPs		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
P BP-BC + SeNPs		0.30	< 0.05	0.11	0.13	0.08

Table 6. Impact of senps and BP-BC on leaf greens, membrane leakage, stomatal conductivity, photosynthetic rate, and proline concentration in barley plants subjected to Pb-polluted saline soils.

Treatments	Dose	Mn-SOD	APX	CAT
BP-BC (t ha^{-1})	0	2.59a ± 0.40	3.70a ± 0.30	3.70a ± 0.30
	5	1.89b ± 0.30	2.89b ± 0.40	2.50b ± 0.18
	10	1.20c ± 0.40	2.09c ± 0.29	1.50c ± 0.11
SeNPs (mg L^{-1})	0	2.40a ± 0.59	3.29a ± 0.70	3.20a ± 0.20
	10	1.8b ± 0.59	2.80b ± 0.69	2.4b ± 0.10
	20	1.70b ± 0.59	2.70b ± 0.70	2.20b ± 0.70
BP-BC + SeNPs	0	3.40a ± 0.59	4.29a ± 0.70	4.20a ± 0.20
	10	2.8b ± 0.59	3.80b ± 0.69	3.4b ± 0.10
	20	2.70b ± 0.59	3.70b ± 0.70	3.20b ± 0.70
p BP-BC		< 0.01	< 0.01	< 0.01
p SeNPs		< 0.01	< 0.01	< 0.01
p BP-BC + SeNPs		0.15	0.30	< 0.10

Table 7. Impact of senps and BP-BC on the Mn-SOD, APX and CAT gene expression in barley plants subjected to Pb-polluted saline soils.

levels (28.30-32.71%) and membrane leakage (17.50-20.23%), while simultaneously improving leaf greenness (20.01–23.29%), stomatal conductance (18.06–22.14%) and photosynthetic rate (30.18–33.18%) in comparison to control group (SeNPs 0 mg L^{-1}).

Table 6 demonstrates that simultaneous use of SeNPs and BP-BC did not produce a significant interactive effect on stomatal conductance, leaf greenness, and membrane leakage and proline levels in leaves, except for photosynthesis rate, which displayed distinct interaction between treatments. The simultaneous use of SeNPs (25 mg L^{-1}) and BP-BC (10 t ha^{-1}) led to significant rise in rate of photosynthesis (140.68%) than control group.

Transcriptional analysis of antioxidants

The SeNPs and BP-BC utilization markedly reduced Mn-SOD, APX, and CAT gene expression in barley exposed Pb-polluted saline soils (Table 7). The findings demonstrate that BP-BC application significantly reduced gene expression degrees of Mn-SOD, APX, and CAT more effectively than SeNPs foliar application. BP-BC application (5 t ha^{-1} and 10 tons ha^{-1}) led to down-regulations in CAT (29.41–59.48%), Mn-SOD (27.22–56.24%), and APX (19.74–9.41%) gene expression, in comparison to control (BP-BC 0 t ha^{-1}). Likewise, SeNPs foliar application at dosages of 10 and 20 mg L^{-1} caused in decreases in CAT (28.30-32.11%), Mn-SOD (28.14–31.27%), and APX (17.50-20.09%) gene expression, compared to control (SeNPs 0 mg L^{-1}). BP-BC application (10 tons ha^{-1}) significantly downregulated Mn-SOD, APX and CAT gene expression in barley plants subjected to Pb-polluted saline soils, surpassing the effects of other treatments, even the control.

Yield parameters

Exposure of barley crop to Pb-polluted saline-nature soil led to reduction in yield characteristics, such as total grain yield, per spike grain numbers, and 1000-grain weight. The SeNPs and BP-BC utilization, whether separately or in conjunction, markedly improved these characteristics in barley plants cultivated in Pb-polluted saline-nature soil. Results in Table 8 demonstrate that use of BP-BC resulted in notable enhancements in total grain yield, per spike grain numbers, and 1000-grain weight relative to SeNPs single application. In comparison

Treatments	Dose	Per spike grain numbers	Grain yield (t ha^{-1})	1000-grain weight (g)
BP-BC (t ha^{-1})	0	40.20c ± 1.59	3.50c ± 0.39	39.27c ± 1.40
	5	45.1b ± 2.6	4.30b ± 0.29	47.50b ± 1.90
	10	50.57a ± 2.40	5.01a ± 0.30	52.13a ± 1.99
SeNPs (mg L^{-1})	0	39.14b ± 2.50	3.80b ± 0.81	44.81b ± 1.30
	10	46.7a ± 2.09	4.39a ± 0.60	48.14a ± 1.80
	20	48.1a ± 2.20	4.50a ± 0.7	48.88a ± 1.90
BP-BC + SeNPs	0	40.14b ± 2.50	4.80b ± 0.81	45.81b ± 1.30
	10	47.7a ± 2.09	5.39a ± 0.60	49.14a ± 1.80
	20	49.1a ± 2.20	5.50a ± 0.7	49.88a ± 1.90
p BP-BC		< 0.01	< 0.01	< 0.01
p SeNPs		< 0.01	< 0.01	< 0.01
p BP-BC + SeNPs		< 0.05	0.15	0.08

Table 8. Impact of senps and BP-BC on the yield-related attributes of barley cultivated in Pb-polluted saline soils.

to the control (BP-BC 0 t ha^{-1}), the BP-BC application (5 t ha^{-1} and 10 tons ha^{-1}) led to enhancements in per spike grains (16.40–30.60%), grain yield (23.22–39.11%), and 1000-grain weight (15.50–27.30%), respectively. Furthermore, SeNPs foliar application (120 and 20 mg L^{-1}) markedly increased per spike grain numbers (10.22–11.88%), 1000-grain weight (8.60–9.41%), and grain yield (17.01–18.21%) in comparison to control (SeNPs 0 mg L^{-1}). Table 8 demonstrates that simultaneous use of SeNPs and BP-BC did not substantially influence 1000-grain weight; nevertheless, a significant interaction with grain yield was observed. The use of BP-BC (10 t ha^{-1}) mixed with SeNPs (25 mg L^{-1}) caused considerable rise in grain yield (80.20%) relative to control.

Discussion

Inhibition of growth in soil influenced by Pb and salinity

There is scant information regarding the application of *Broussonetia papyrifera*-derived biochar (BP-BC) amended soil combined with SeNPs to alleviate the impacts of saline in co-polluted soil. Soil salinity, in conjunction with toxic metal pollution like Pb, can significantly hinder plant photosynthetic capacity by disrupting chloroplast function, altering pigment composition, and generating oxidative stress. High salt levels can lead to ionic imbalance, osmotic stress, and reduced stomatal conductance, limiting CO_2 assimilation. Pb toxicity can replace essential ions in photosynthetic enzymes, damage photosynthetic apparatus, and induce oxidative damage, further compromising photosynthetic efficiency. The combined stress can exacerbate damage to photosynthetic machinery, ultimately reducing plant growth and productivity[59]. In the present research, unamended control plots had deteriorated soil quality, evidenced by reductions in biological, and physiochemical characteristics. This degradation led to enhanced accumulation and absorption of Pb and Na in different plant tissues, resulting in disrupted biochemical and physiological functions in barley plants. Consequently, growth and yield of plants were markedly diminished, by results of[60]. The combined stress of lead and salinity in soil can inhibit plant growth by disrupting cellular homeostasis, nutrient uptake, and metabolic processes. Pb toxicity can alter enzyme activity, damage cell membranes, and induce oxidative stress, while high salinity can cause osmotic stress, ion imbalance, and reduced water availability. The synergistic effects can lead to reduced root and shoot growth, impaired photosynthesis, and decreased biomass production. Additionally, Pb can accumulate in plant tissues, further exacerbating toxicity and growth inhibition. This combined stress can ultimately compromise plant survival and productivity in affected soils.

BP-BC and senps improved soil properties influenced by Pb and salinity

This study shows enhancements in the biological, and physiochemical characteristics of soil when BP-BC-treated soil is combined with SeNPs as contaminants barrier, in contrast to individual application and control groups. These improvements are associated with efficacy of the combined treatments in decreasing Pb concentration and exchangeable Na in Pb-polluted and saline soils. The combination of biochar and selenium nanoparticles (SeNPs) can enhance the biological and physicochemical characteristics by synergistically improving soil fertility, structure, and microbial activity. Biochar can increase soil's water-holding capacity, aeration, and cation exchange capacity, while SeNPs can stimulate beneficial microbial growth and enhance enzyme activity. Biochar's porous structure can also adsorb and retain nutrients, reducing leaching and making them available to plants, while SeNPs can provide essential selenium micronutrient, promoting plant growth and stress tolerance. This combination can create a favorable environment for plant growth, improve nutrient cycling, and enhance overall soil health[61]. SeNPs, with their antioxidant and antimicrobial properties, can further stimulate beneficial microbial activity, promote plant growth, and enhance soil's overall biological health. The synergistic interaction between biochar and SeNPs may also improve soil's physicochemical properties, such as pH buffering capacity, CEC, and nutrient availability, ultimately leading to improved soil fertility and plant productivity. This combined approach can contribute to sustainable agriculture and ecosystem restoration by promoting soil health and resilience[62]. Furthermore, elevated microbial activity and soil enzyme indicate improved availability of nutrients for microbes in soils amended with BP-BC and enriched with SeNPs can be attributed to the improved availability of nutrients and a favorable microenvironment. BP-BC provides a habitat for microorganisms, increasing their abundance and diversity, while its porous structure adsorbs and retains nutrients, making them available to microbes. SeNPs further stimulate microbial growth and enzyme activity, potentially by providing essential selenium micronutrient, enhancing redox reactions, and promoting beneficial microbial interactions. SThis synergy enhances nutrient cycling, decomposition, and overall soil health. These results corroborate past studies indicating that organic supplements, such as biochar, boost soil health, increase availability of nutrients, and are important for preserving membrane integrity and cell wall[63]. The elevated microbial activity and soil enzyme levels after biochar and SeNPs application can be attributed to the creation of a favourable soil environment. Biochar provides a habitat for microorganisms, increasing their abundance and diversity, while its porous structure and high surface area support nutrient retention and exchange[64]. SeNPs stimulate microbial growth and activity by providing essential selenium nutrients, which are incorporated into selenoproteins that play crucial roles in antioxidant defense and redox reactions. SeNPs also enhance enzyme production by inducing the expression of genes involved in enzyme synthesis, and promote beneficial microbial interactions by modulating signaling pathways and biofilm formation. Additionally, SeNPs' small size and high surface area allow for efficient interaction with microorganisms, facilitating the uptake of selenium and promoting microbial metabolism, ultimately leading to increased microbial activity and improved soil health. As a result, soil enzyme activities, such as those involved in nutrient cycling, increase, indicating improved soil health, fertility, and ecosystem are functioning. This synergistic effect can lead to enhanced nutrient availability, plant growth, and overall soil sustainability[65]. The rise in microbial function also enhances soil N fixation, facilitating the growth of plants[66]. BP-BC reduced soil pH likely due to its acidic functional groups and buffering capacity. BP-BC's high surface area and porosity improved soil structure by increasing water infiltration, aeration, and root growth. The biochar's porous structure also enhanced soil aggregation by acting as a binding agent, improving soil stability and water retention. Additionally, the increased surface area and porosity of BP-BC improved soil permeability, allowing for better water and air movement, and facilitating root growth and microbial activity, ultimately enhancing soil health and fertility. These alterations subsequently improved drainage, decreased sodium levels, diminished salinity in soil, and boosted physical soil characteristics, resulting in improved soil health[67]. BP-BC markedly enhanced hydraulic conductivity and CEC, consequently increasing the availability of nutrients in soil, the application of biochar and SeNPs can markedly enhance hydraulic conductivity and CEC in soil, due to biochar's porous structure and high surface area, which increases water retention and infiltration, thereby improving hydraulic conductivity. Biochar's negative charge also contributes to increase CEC, allowing it to retain and exchange cations, making nutrients more available to plants. SeNPs may further enhance soil structure and aggregation, potentially improving water movement and retention, while their interaction with biochar may synergistically enhance soil's physical and chemical properties, ultimately benefiting plant growth and soil health[68]. Additionally, biochar's high CEC enables it to retain and exchange nutrients, making them more available to plants. SeNPs may further enhance these effects by promoting soil aggregation and structure, facilitating water and nutrient movement. The combined application of biochar and selenium nanoparticles (SeNPs) creates a synergistic effect by integrating biochar's structural benefits, such as improved soil aeration, water retention, and cation exchange capacity, with SeNPs' biochemical benefits, including enhanced microbial activity and enzyme production. This synergy improves soil's physical properties, like aggregation and porosity, and chemical properties, like nutrient availability and retention. As a result, nutrient uptake by plants is increased, leading to improved soil fertility and productivity. The interaction between biochar and SeNPs fosters a favorable environment for plant growth, enhancing root development, and ultimately promoting plant health and yield[69]. Moreover, the enhanced microbial activity, evidenced by microbial biomass C and CO_2 influx, along with increased alkaline phosphatase and dehydrogenase activity, resulted in an enhancement of beneficial soil microorganisms. These microorganisms excrete polysaccharides that enhance soil OM and quality, facilitating Pb immobilization[70].

The enhanced soil productivity in biochar-amended soils can be attributed to biochar's ability to immobilize Pb, reduce salt stress, and improve soil's physical and chemical properties. Biochar's high cation exchange capacity and surface area enable it to adsorb Pb, reducing its bioavailability and toxicity to plants. Additionally, biochar improves soil structure, aeration, and water retention, mitigating salt-induced osmotic stress. By creating a more favorable environment, biochar promotes microbial activity, nutrient cycling, and plant growth, ultimately leading to improved soil quality and productivity in Pb-polluted and saline environments. The soil pH, an essential measure of soil health, diminished from moderate alkaline (8.1–8.4) to mildly alkaline (7.6–7.8) in the soil treated with BP-BC. The decrease in soil pH from moderate alkaline to mildly alkaline in biochar-treated soil can be attributed to biochar's buffering capacity and its effect on soil ion dynamics. Biochar's high surface area and cation exchange capacity can adsorb and retain alkaline cations, such as calcium and magnesium, reducing their concentration in the soil solution and subsequently lowering the pH. Additionally, biochar can release acidic functional groups, contributing to a decrease in soil pH. This shift towards a mildly alkaline pH can create a more favorable environment for plant growth and microbial activity, potentially enhancing soil fertility and overall health[71]. Soil pH plays a crucial role in influencing metal mobility and speciation, as it affects the chemical forms and bioavailability of metals. A decrease in soil pH (increasing acidity) generally increases the mobility and bioavailability of most metals, such as lead, cadmium, and zinc, by promoting their dissolution and release from soil particles. Conversely, an increase in soil pH (increasing alkalinity) can lead to the precipitation of metals, reducing their mobility and bioavailability. Additionally, soil pH influences the speciation of metals, altering their chemical forms and reactivity, which can impact their toxicity and uptake by plants, ultimately affecting environmental and human health risks associated with metal contamination. These results align with recent research indicating that char can Pb immobilize via its positive charged surface functional groups[72,73].

In unamended plots, elevated Pb levels diminished soil quality; impeding growth of seedlings caused by a decrease in biological attributes, including diminished microbial biomass carbon, reduced microbial populations, and reduced enzymatic activity, consistent with other research findings[74]. The elevated carbon (C) content and organic carbon (OC) levels in BP-BC-treated soils can be attributed to biochar's stable carbon structure, which resists decomposition and persists in the soil, contributing to long-term carbon sequestration. This increased C content provides a food source for microorganisms, stimulating their growth and activity, and enhancing enzymatic biological activity. The improved OC levels also support the formation of stable soil aggregates, enhance nutrient retention, and promote beneficial microbial interactions, ultimately leading to improved soil fertility, structure, and overall health[75]. An inverse correlation was discovered between OC and Pb levels in polluted soils. The BP-BC additions promoted oxygen supply and soil aeration, reduced soil pH, improved soil chemical characteristics, and increased availability of nutrients, as documented in other studies[76].

Salinity was found to enhance Pb toxicity and mobility in polluted soils by increasing solubility and bioavailability of Pb through formation of soluble Pb complexes with chloride ions. High salt concentrations can also disrupt soil structure, reduce sorption sites, and increase Pb desorption, making it more mobile and available for plant uptake. Additionally, salinity-induced changes in soil pH and ionic strength can further influence Pb speciation and mobility, exacerbating its toxic effects on plants and microorganisms and ultimately compromising soil health and ecosystem function. Consequently, the research focused on alleviating soil salinity, which is directly linked to a decrease in Pb levels in contaminated soils. Biochar, produced at high temperatures, exhibits considerable specific surface area and notable cation exchange capacity, resulting in reduced exchangeable sodium percentage (ESP) and electrical conductivity. The negative charges on biochar functional groups hindered rhizosphere sodium ions, thereby diminishing Pb accessibility by increasing its sorption onto aqueous surfaces, beyond soil solutions[77].

The results of this research demonstrated that the use of SeNPs enhanced the Pb and Na accumulation in cell of leaves while diminishing their levels in organelle bodies, likely due to SeNPs' role in modulating plant defense mechanisms and altering ion distribution. SeNPs may induce the production of cell wall components, such as lignin and pectin, which can bind and sequester Pb and Na, restricting their entry into organelles and reducing toxicity. This compartmentalization strategy allows plants to tolerate high levels of Pb and Na, minimizing damage to sensitive organelles and maintaining cellular function, ultimately promoting plant growth and survival under stress conditions. Similarly, Pb accumulated in the cell walls of the roots, whilst its soluble fraction within the root was diminished[78]. The elevated density of negative charged active groups in cell wall was seen to bind Na and immobilize Pb, hence restricting their transit throughout plant. Furthermore, an antagonistic link between Se and both Na and Pb was noted[79]indicating that elevating Se levels via spraying may diminish Na and Pb levels in plant tissues. Consequently, due to their unique properties, including small size, high surface area-to-volume ratio, and increased SSA. These nanoscale dimensions enable SeNPs to interact more efficiently with plant cells, facilitating uptake and distribution of selenium. The increased SSA of SeNPs also enhances their reactivity, allowing for improved bioavailability and efficacy of selenium, which can stimulate plant growth, enhance stress tolerance, and promote beneficial biochemical reactions, ultimately contributing to improved plant health and productivity[80].

SeNPs, measuring between 1 and 100 nm, alleviate detrimental impacts of abiotic stresses on plants by enhancing antioxidant defense systems, improving stress tolerance, and regulating biochemical pathways. SeNPs' small size allows for efficient uptake and distribution within plant cells, where they can scavenge reactive oxygen species, modulate hormone signaling, and enhance enzyme activity. This leads to improved plant growth, increased photosynthetic efficiency, and enhanced stress resilience, ultimately mitigating the negative effects of abiotic stresses such as drought, salinity, and heavy metal toxicity on plant productivity and health[81]. Consequently, employing SeNPs in place of conventional Se fertilizers can improve Se availability to satisfy nutritional requirements of barley crops, while simultaneously decreasing Na and Pb uptake[82]. The application of SeNPs in this manner facilitated moisture retention in both roots and leaves, enhanced nutrient absorption, and increased canopy growth by improving photosynthetic efficiency, chlorophyll concentration, stomatal conductance, and gas exchange. As a result, there was an increase in photoassimilate translocation to grains in saline and Pb-contaminated soils[83]. Selenium is essential for respiration as it facilitates the synthesis of enzymes associated with this process[82]. Furthermore, our research demonstrated that Se marginally enhanced soil characteristics in vicinity of plant rhizosphere likely due to its role in promoting plant growth and root development, which in turn improves soil structure and fertility. Selenium can stimulate beneficial microbial activity, enhance root exudation, and increase soil enzyme activity, leading to improved nutrient cycling and availability. Additionally, Se's antioxidant properties may help mitigate oxidative stress in the rhizosphere, creating a more favorable environment for plant-microbe interactions and ultimately contributing to improved soil health and fertility in the rhizosphere. Nonetheless, its synergistic application with biochar-treated soil produced a markedly greater benefit than its individual use. These results validate previous research, highlighting the substantial importance of SeNPs, hence reinforcing outcomes of the present study[84].

SeNPs are absorbed via leaf endocytosis or epidermis and migrate through apoplastic routes into phloem, then aggregate in cell walls of root and affect rhizosphere[85]. This process improves the uptake of nutrients by root cells and reduces Na and Pb uptake, hence facilitating growth. Selenium is vital for plant growth, serving as a co-factor for enzymes that facilitate tryptophan production, which subsequently generates auxin hormone, required for crop growth[86]. Recent studies have demonstrated that SeNPs can enhance the absorption of essential minerals for plant growth and metabolism, even in adverse soil conditions that would normally impede nutrient uptake[82].

Research, including[87]demonstrates that biochar use, abundant in N and C, binds Na ions and immobilizes Pb, transforming them into insoluble forms within soil solution. This diminishes their uptake via roots and restricts their movement via xylem to crop tissues, thereby decreasing BAC, TF, and BCF in Pb-polluted and saline soils. These results indicate that biochar-treated soil, abundant in OM, is more proficient at binding Na and immobilizing Pb than saline soil contaminated with Pb and devoid of biochar. Moreover, SeNPs markedly diminished Pb absorption in barley plants through augmenting the antioxidant capacity and lowering free radical concentrations, which aligns with their influence on Mn-SOD, APX, and CAT.

Moreover, SeNPs mitigated Pb toxicity in barley plants by enhancing antioxidant defense systems, regulating Pb uptake and distribution, and modulating stress-related gene expression. SeNPs likely scavenged reactive oxygen species, reducing oxidative stress and damage to plant cells. Additionally, SeNPs may have restricted Pb translocation to sensitive tissues, sequestered Pb in less sensitive cellular compartments, and improved plant nutrient balance, ultimately promoting plant growth and tolerance to Pb stress. This mitigation strategy enables barley plants to cope with Pb-induced stress, maintaining cellular homeostasis and functionality[22]. Research has demonstrated that SeNPs reduced Pb accumulation in many plant tissues while enhancing stability of cell membranes likely by regulating Pb uptake, transport, and sequestration. SeNPs may have increased the expression of genes involved in Pb exclusion or compartmentalization, restricting Pb entry into sensitive tissues or sequestering it in vacuoles. Additionally, SeNPs' antioxidant properties helped maintain cell membrane integrity by reducing lipid peroxidation and enhancing membrane-bound enzyme activity, thereby protecting plant cells from Pb-induced oxidative damage and maintaining cellular function and homeostasis[88]. Furthermore, SeNPs improved barley plant health by decreasing Pb absorption and toxicity through mechanisms such as Pb immobilization, restriction of Pb uptake, and enhancement of antioxidant defenses. SeNPs may have also promoted the expression of genes involved in Pb exclusion or sequestration, reducing Pb translocation to sensitive tissues. Concurrently, SeNPs increased Se uptake, likely due to their high bioavailability and efficient absorption by plant roots, allowing for beneficial Se accumulation and utilization by the plant, ultimately enhancing plant growth, stress tolerance, and nutritional quality[89,90]. The data indicate that nutrient buildup transpires through ongoing root absorption via xylem and remobilization via phloem from vegetative portions to grains, eventually enhancing plant output and characteristics[88].

BP-BC and senps improved biochemical, physiological and barley yield characteristics in saline-impacted and Pb-polluted soils

Our results indicated that the simultaneous application of BP-BC-treated soil with SeNPs, in contrast to control and single use, markedly diminished uptake and accumulation of Na and Pb in barley plants by synergistically enhancing soil properties and plant defense mechanisms. BP-BC likely immobilized Pb and reduced Na availability through adsorption and complexation, while SeNPs enhanced antioxidant defenses and regulated ion uptake, restricting Na and Pb translocation to sensitive tissues. This combined approach improved soil fertility, reduced metal bioavailability, and promoted plant stress tolerance, ultimately mitigating the adverse effects of Pb pollution and salinity on barley plant growth and productivity[87]. The co-addition resulted in the elevated activity of APX and CAT, which aligned with the augmented antioxidant genes expression Mn-SOD, APX, and CAT with reduction in oxidative stress markers such as EL, H_2O_2 and MDA likely triggered a synergistic response, enhancing plant stress tolerance and promoting cellular homeostasis by mitigating oxidative damage and maintaining redox balance. These enhancements ultimately led to superior nutritional uptake such as Zn, Mg, and K, and enhanced gas exchange metrics such as Tr, Pn, Ci, Gs, and SPAD in barley plants[91]. These findings corroborate previous research demonstrating that BP-BC increases nutrient availability in the rhizosphere, enhances root absorption, accelerates nutrient translocation through xylem vessels, and promotes Mg and K accumulation in leaves, while reducing Na levels. This augmentation positively affects stomatal conductance, photosynthesis, and carbon dioxide levels by increasing chlorophyll concentration, leading to superior plant development and growth compared to unamended plants in Pb-contaminated and saline soils[92].

The research indicated a significant association between BP-BC treatment and decreased absorption of Na and Pb through the roots, along with their resulting buildup in plant leaves under these circumstances[93]. The elevated potassium and magnesium levels in leaves, seen with BP-BC treatment, significantly enhance plant resistance to environmental stresses, as documented in prior study[94]. Unamended soils exhibited significant oxidative damage, characterized by elevated levels of H_2O_2 and MDA, resulting in increased electrolyte leakage, generation of reactive oxygen species, and damage of cell membranes in barley plants cultivated in Pb-polluted and saline soils[95]. In contrast, BP-BC treatment markedly diminished oxidative stress markers, likely by adsorbing and immobilizing reactive oxygen species (ROS)-inducing substances, such as heavy metals, and improving soil properties. Biochar's high surface area and functional groups may have also directly scavenged ROS, reducing oxidative stress. Additionally, biochar may have stimulated plant antioxidant defense systems, enhancing the activity of enzymes such as superoxide dismutase, peroxidase, and catalase, which help detoxify ROS, ultimately protecting plants from oxidative damage and promoting cellular homeostasis[96]. The biochar treatment's diminishment of oxidative stress markers can be attributed to its ability to modulate antioxidant activity. Biochar's surface functional groups and porous structure can scavenge reactive oxygen species (ROS) and reduce oxidative stress. The increased activity of antioxidant enzymes such as ascorbate peroxidase (APX) and catalase (CAT) suggests that biochar triggers a protective response in plants, enhancing their antioxidant defense system. APX and CAT work in tandem to detoxify hydrogen peroxide, a key ROS, thereby reducing oxidative damage. By mitigating oxidative stress, biochar treatment can promote plant growth, improve stress tolerance, and enhance overall plant health. The correlation between reduced oxidative stress markers and increased antioxidant activity highlights biochar's potential as a soil amendment to support plant well-being[94]. Biochar facilitated the neutralization of reactive oxygen species (ROS) in plant cells by enhancing the activity of antioxidant enzymes, such as catalase, which converts hydrogen peroxide into water and oxygen. This enzymatic reaction reduces oxidative stress, diminishing lipid peroxidation and protein degradation. By mitigating ROS-induced damage, biochar promotes cellular homeostasis, allowing plants to allocate resources towards growth and development, ultimately enhancing crop growth, yield, and productivity. Biochar's surface functional groups and nutrient supply may also contribute to improved plant nutrition and stress tolerance.

Chlorophyll levels in leaves serve as a crucial marker of plant capacity to flourish in Pb-polluted and saline soils[97]. The elevated levels of chlorophyll noted in the present research likely by improving nutrient availability, particularly nitrogen, iron, and magnesium, essential for chlorophyll synthesis. Biochar's adsorption properties may have also reduced nutrient leaching, ensuring a steady supply of these critical nutrients. Additionally, biochar's impact on soil pH and microbial activity may have enhanced root growth and function, allowing for increased nutrient uptake and chlorophyll production, ultimately improving photosynthetic efficiency and plant growth. This increase in chlorophyll content enables plants to capture more light energy, driving photosynthesis and supporting overall plant productivity[98]. Furthermore, SeNPs enhanced antioxidant function, diminished reactive oxygen species levels, and fortified integrity of cell membranes by decreasing electrolyte leakage. The enhancements were correlated with elevated Zn, Mg, and K in Pb-polluted and saline soils[99]. A significant enhancement in barley's biochemical and physiological traits was observed when BP-BC was combined with SeNPs, as opposed to separate applications. This was observed in grain yield and yield-related variables under these extreme conditions[92]. Our findings indicated that optimal enhancement of soil characteristics and plant performance was attained through synergistic use of BP-BC-treated soil and SeNPs, in contrast to alone use of BP-BC or SeNPs foliar treatment[100]. Results indicate that nutrient buildup in plants results from ongoing root absorption by xylem and subsequent remobilization via phloem, hence improving crop output and yield-related characteristics, particularly in semi-arid environments with saline soils[101–104].

Conclusion

The synergistic application of SeNPs and BP-BC has shown a significant positive impact on the remediation of Pb-contaminated salty soils, improved barley crop efficiency by reducing Pb bioavailability, improving soil quality, and increasing plant resistance to oxidative stress. The findings suggest that this environmentally friendly approach could serve as a viable alternative to conventional soil treatments in agricultural regions affected by salt and lead contamination. Future research should focus on modifying these treatments for various crops and soil types, examining their long-term effects on soil microbial communities, and assessing the broader environmental implications of SeNPs application in agriculture. Future research should investigate synergistic effects of SeNPs and biochar derived from *Broussonetia papyrifera* on remediation of lead-contaminated saline soils during barley cultivation. This could include optimizing the application rates of SeNPs and biochar, as well as examining their combined influence on soil microbial communities, plant growth and lead immobilization. Examining long-term stability and possible environmental hazards of these modifications is essential. Furthermore, expanding this technology for field applications and evaluating its economic viability will be crucial for actual implementation. Furthermore, investigating impacts of SeNPs and biochar on additional crops and contaminants could expand the parameters of this remediation approach, aiding in formulation of sustainable and efficient techniques for rehabilitating degraded soils.

While selenium nanoparticles (SeNPs) show promise in enhancing plant growth and stress tolerance, there are limitations to their use. Potential drawbacks include the narrow therapeutic window between beneficial and toxic effects of selenium, potential environmental accumulation and toxicity, and variability in SeNPs' physicochemical properties affecting their efficacy and stability. Moreover, the long-term impacts of SeNPs on plant and soil health, as well as potential effects on non-target organisms, require further investigation. Additionally, scalability, cost-effectiveness, and regulatory frameworks for SeNPs' use in agriculture need to be addressed to ensure their safe and sustainable application.

Future research directions for this topic could involve conducting long-term field studies to assess the sustainability and effectiveness of *Broussonetia papyrifera*-derived biochar and selenium nanoparticles in remediating lead-polluted saline soils. Additionally, optimizing application rates, scaling up the remediation approach, and evaluating economic feasibility and environmental impacts would be crucial. Investigating underlying mechanisms of biochar and selenium nanoparticles in immobilizing lead and promoting plant growth would also provide valuable insights, ultimately contributing to sustainable management of lead-polluted saline soils and promoting safe crop cultivation.

Data availability

All the raw data in this research can be obtained from the corresponding authors upon reasonable request.

Received: 22 May 2025; Accepted: 16 July 2025
Published online: 23 July 2025

References

1. Men, D., Li, X., Zhu, X. & Zhao, C. Heavy metal (loid) s pollution in soils of a typical agricultural and rural area: source apportionment and derived risk quantification. *Environ. Monit. Assess.* **196** (12), 1224 (2024).
2. Mai, X. et al. *Research Progress on the Environmental Risk Assessment and Remediation Technologies of Heavy Metal Pollution in Agricultural Soil* (Journal of Environmental Sciences, 2024).
3. Feng, J. J., Liao, J. X., Jiang, Q. W. & Mo, L. Heavy metal contamination of vegetables in china: status, causes, and impacts. *Environ. Sci. Pollut. Res.* **32** (2), 864–873 (2025).
4. Sultana, S., Sultana, N., Moniruzzaman, M., Dastagir, M. R. & Hossain, M. K. Unmasking heavy metal contamination: tracing, risk estimating and source fingerprinting from coastal sediments of the Payra river in Bangladesh. *Mar. Pollut. Bull.* **211**, 117455 (2025).
5. Datta, K., Chakraborty, S. & Roychoudhury, A. Management of soil, waste and water in the context of global climate change. In *Environmental Nexus for Resource Management* (1–26). (CRC, 2025).
6. Qureshi, A. S. & Perry, C. Managing water and salt for sustainable agriculture in the indus basin of Pakistan. *Sustainability* **13** (9), 5303 (2021).

7. Ondrasek, G. & Rengel, Z. Environmental salinization processes: detection, implications & solutions. *Sci. Total Environ.* **754**, 142432 (2021).
8. Murtaza, G. et al. Synergistic effects of Allantoin and Achyranthes japonica-biochar profoundly alleviate lead toxicity during barley growth. *Ecotoxicol. Environ. Saf.* **290**, 117784 (2025).
9. Ahmad, W. et al. Cereal crops in the era of climate change: An overview. Environment, Climate, Plant and Vegetation Growth, 609–630. (2024).
10. Murtaza, G., Ahmed, Z., Iqbal, R. & Deng, G. Biochar from agricultural waste as a strategic resource for promotion of crop growth and nutrient cycling of soil under drought and salinity stress conditions: a comprehensive review with context of climate change. *J. Plant Nutr.*, 1–52. (2025).
11. Shah, S. S. et al. Impact of irrigation, fertilizer, and pesticide management practices on groundwater and soil health in the rice–wheat cropping system—a comparison of conventional, resource conservation technologies and conservation agriculture. *Environ. Sci. Pollut. Res.* **32** (2), 533–558 (2025).
12. Singh, A. et al. Addressing abiotic stresses and advancing SDGs by Biochar for sustainable agriculture and environmental restoration. *Egypt. J. Soil Sci.* **65** (1), 463–489 (2025).
13. Wang, Y. et al. Unraveling how Fe-Mn modified Biochar mitigates Sulfamonomethoxine in soil water: the activated biodegradation and hydroxyl radicals formation. *J. Hazard. Mater.* **465**, 133490 (2024).
14. Kamran, M. et al. An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation. *Int. J. Mol. Sci.* **21** (1), 148 (2019).
15. Chandwani, S., Kayasth, R., Naik, H. & Amaresan, N. Current status and future prospect of managing lead (Pb) stress through microbes for sustainable agriculture. *Environ. Monit. Assess.* **195** (4), 479 (2023).
16. Seow, Y. X. et al. A review on Biochar production from different biomass wastes by recent carbonization technologies and its sustainable applications. *J. Environ. Chem. Eng.* **10** (1), 107017 (2022).
17. Naeem, M. B. et al. Improving maize yield and drought tolerance in field conditions through activated Biochar application. *Sci. Rep.* **14** (1), 25000 (2024).
18. Wang, F., Qi, X., Zhang, H. & Yang, Z. Innovative molten salt techniques for biomass valorization: transforming biomass into advanced carbon materials. *Carbon* **234**, 119999 (2025).
19. Yang, Y. et al. Unlocking mechanisms for soil organic matter accumulation: carbon use efficiency and microbial necromass as the keys. *Glob. Change Biol.*, **31**(1), e70033. (2025).
20. Antonangelo, J. A., Sun, X. & Eufrade-Junior, H. D. J. Biochar impact on soil health and tree-based crops: a review. *Biochar* **7** (1), 51 (2025).
21. Lu, Y. et al. In-situ synthesis of lanthanum-coated sludge Biochar for advanced phosphorus adsorption. *J. Environ. Manage.* **373**, 123607 (2025).
22. Cao, Y., Zhao, Z., Zhang, D., Liang, A. & Li, L. Machine learning-based soil improvement techniques and their optimization of farmland soil water retention capacity. *Adv. Resour. Res.* **5** (2), 919–945 (2025).
23. Lin, J. et al. Effect of degradable microplastics, Biochar and their coexistence on soil organic matter decomposition: A critical review. *TRAC Trends Anal. Chem.* **183**, 118082 (2025).
24. Gödekmerdan, E. & Baştabak, B. Biochar production methods and their diverse applications in improving plant production system to achieve agricultural sustainability. In *Biochar Ecotechnology for Sustainable Agriculture and Environment* (157–179). (Elsevier, 2025).
25. Yin, Y. et al. Underestimated sequestration of soil organic carbon in China. *Environ. Chem. Lett.* **23** (2), 373–379 (2025).
26. Zaman, W., Ayaz, A. & Park, S. Nanomaterials in agriculture: A pathway to enhanced plant growth and abiotic stress resistance. *Plants* **14** (5), 716 (2025).
27. Wu, X. & Zhao, Y. A novel heat pulse method in determining effective thermal properties in frozen soil. *Water Resources Res.*, **60** (12), e2024WR037537. (2024).
28. Noreen, S. et al. Advancing plant resilience against microplastics and metals through nanotechnology. *BioNanoScience* **14** (2), 2065–2079 (2024).
29. Jahan, A., Sarkar, M. I. U., Naher, U. A., Biswas, J. C. & Islam, A. Effect of sterile rice spikelets derived Biochar amendment on nutrient leaching and availability in paddy soil under continuous standing water. *Geol. Ecol. Landscapes.* **8** (4), 574–582 (2023).
30. Roy, T. K., Islam, M. S., Mahiddin, N. A., Hossain, S. A., Biswas, T., Antu, U. B.,… Ismail, Z. (2025). Application of nanoparticles (NPs) to ameliorate abiotic stress in economically important crop species: a potential review. *J. Crop Health* 77(1), 1–20.
31. Maljaee, H., Madadi, R., Paiva, H., Tarelho, L. & Ferreira, V. M. Incorporation of Biochar in cementitious materials: A roadmap of biochar selection. *Constr. Build. Mater.* **283**, 122757 (2021).
32. Imran & Ortas, I. Nano biotechnology: exploring the potential of nano materials in enhancing crop health and combating abiotic stress. *Commun. Soil Sci. Plant Anal.*, 1–20. (2024).
33. Dakal, T. C., Bhushan, R., Dhakar, R. & Kumar, A. Classification and applications of Bio-nanomaterials. *Bio-Nanomaterials Environ. Remediation: Industrial Appl.*, 47–73. (2025).
34. Alharbi, M., waqas Iqbal, M., Al-Hadeethi, Y. & Hussein, M. A. Photocatalytic activity of composite material-based nylon 6/sulfur NPs and reinforced carbon nanotubes. *Diam. Relat. Mater.* **151**, 111859 (2025).
35. Yuan, Z. et al. Research progress on the physiological mechanism by which selenium alleviates heavy metal stress in plants: a review. *Agronomy* **14** (8), 1787 (2024).
36. Somagattu, P., Chinnannan, K., Yammanuru, H., Reddy, U. K. & Nimmakayala, P. *Selenium Dynamics in Plants: Uptake, Transport, Toxicity, and Sustainable Management Strategies*175033 (Science of The Total Environment, 2024).
37. Garousi, F. Toxicity of selenium, application of selenium in fertilizers, selenium treatment of seeds, and selenium in edible parts of plants. *Acta Universitatis Sapientiae Aliment.* **10** (1), 61–74 (2017).
38. 0 Zahedi, S. M., Abdelrahman, M., Hosseini, M. S., Hoveizeh, N. F. & Tran, L. S. P. Alleviation of the effect of salinity on growth and yield of strawberry by foliar spray of selenium-nanoparticles. *Environ. Pollut.* **253**, 246–258 (2019).
39. Alessandrino, L. Effect of Biochar application on physiochemical properties and nitrate degradation rate in a siliciclastic riverine sandy soil. *Environ. Earth Sci.* **83** (19), 1–13 (2024).
40. Abdollahnia, M., Makhdoumi, A., Mashreghi, M. & Eshghi, H. Exploring the potentials of halophilic prokaryotes from a solar saltern for synthesizing nanoparticles: the case of silver and selenium. *PLoS One*, **15**(3), e0229886. (2020).
41. Corwin, D. L. & Yemoto, K. Salinity: electrical conductivity and total dissolved solids. *Soil Sci. Soc. Am. J.* **84** (5), 1442–1461 (2020).
42. Cymes, I. & Glińska-Lewczuk, K. The use of water quality indices (WQI and SAR) for multipurpose assessment of water in dam reservoirs. *J. Elementology*, **21**(4). (2016).
43. Keuskamp, J. A., Schmitt, H., Laanbroek, H. J., Verhoeven, J. T. & Hefting, M. M. Nutrient amendment does not increase mineralisation of sequestered carbon during incubation of a nitrogen limited Mangrove soil. *Soil Biol. Biochem.* **57**, 822–829 (2013).
44. Bhattacharyya, S. S., Ros, G. H., Furtak, K., Iqbal, H. M. & Parra-Saldívar, R. Soil carbon sequestration–An interplay between soil microbial community and soil organic matter dynamics. *Sci. Total Environ.* **815**, 152928 (2022).
45. Redel, Y. et al. Fertilizer P uptake determined by soil P fractionation and phosphatase activity. *J. Soil. Sci. Plant. Nutr.* **19**, 166–174 (2019).

46. Manzoor, S., Habib-ur-Rahman, M., Haider, G. et al. Biochar and slow-releasing nitrogen fertilizers improved growth, nitrogen use, yield, and fiber quality of cotton under arid climatic conditions. Environ Sci Pollut Res 29, 13742–13755 (2022).
47. Kizilkaya, R. Nitrogen fixation capacity of Azotobacter spp. Strains isolated from soils in different ecosystems and relationship between them and the Microbiological properties of soils. *J. Environ. Biol.* **30** (1), 73–82 (2009).
48. Sahu, P. K., Singh, D. P., Prabha, R., Meena, K. K. & Abhilash, P. C. Connecting microbial capabilities with the soil and plant health: options for agricultural sustainability. *Ecol. Ind.* **105**, 601–612 (2019).
49. Kader, M., Lamb, D. T., Mahbub, K. R., Megharaj, M. & Naidu, R. Predicting plant uptake and toxicity of lead (Pb) in long-term contaminated soils from derived transfer functions. *Environ. Sci. Pollut. Res.* **23**, 15460–15470 (2016).
50. Jabeen, Z. et al. Physiological and antioxidant responses of cultivated and wild barley under salt stress. *Plant. Soil. Environ.* **66** (7), 334–344 (2020).
51. Zawoznik, M. S., Groppa, M. D., Tomaro, M. L. & Benavides, M. P. Endogenous Salicylic acid potentiates cadmium-induced oxidative stress in Arabidopsis thaliana. *Plant Sci.* **173** (2), 190–197 (2007).
52. Alshaal, T. et al. Strengthen sunflowers resilience to cadmium in saline-alkali soil by PGPR-augmented Biochar. *Ecotoxicol. Environ. Saf.* **280**, 116555 (2024).
53. Szulc, P., Bocianowski, J., Nowosad, K., Zielewicz, W. & Kobus-Cisowska, J. SPAD leaf greenness index: green mass yield indicator of maize (*Zea mays* L.), genetic and agriculture practice relationship. *Plants* **10** (5), 830 (2021).
54. Iqbal, R., Raza, M.A.S., Saleem, M.F. et al. Physiological and biochemical appraisal for mulching and partial rhizosphere drying of cotton. J. Arid Land 11, 785–794 (2019).
55. Filek, M. et al. Characterization of barley leaf tolerance to drought stress by chlorophyll fluorescence and electron paramagnetic resonance studies. *J. Agron. Crop. Sci.* **201** (3), 228–240 (2015).
56. Kumar, R. R. et al. Heterologous expression and characterization of novel manganese superoxide dismutase (Mn-SOD)–A potential biochemical marker for heat stress-tolerance in wheat(Triticum aestivum). *Int. J. Biol. Macromol.* **161**, 1029–1039 (2020).
57. Petriccione, M., Mastrobuoni, F., Zampella, L. & Scortichini, M. Reference gene selection for normalization of RT-qPCR gene expression data from Actinidia deliciosa leaves infected with *Pseudomonas syringae* pv. actinidiae. *Sci. Rep.* **5** (1), 16961 (2015).
58. James, M. et al. A plant trait analysis of responses to grazing in a long-term experiment. *J. Appl. Ecol.* **38** (2), 253–267 (2001).
59. Ansari, M. K. A. et al. Heavy metal stress and cellular antioxidant systems of plants: A review. *Agricultural Reviews*. **45** (3), 400–409 (2024).
60. Aslam, M., Aslam, A., Sheraz, M., Ali, B., Ulhassan, Z., Najeeb, U., Gill, R. A.(2021). Lead toxicity in cereals: Mechanistic insight into toxicity, mode of action,and management. *Front. plant sci.*, 11, 587785.
61. Anyebe, O., Sadiq, F. K., Manono, B. O. & Matsika, T. A. Biochar characteristics and application: effects on soil ecosystem services and nutrient dynamics for enhanced crop yields. *Nitrogen* **6** (2), 31 (2025).
62. An, X., Liu, Q., Pan, F., Yao, Y., Luo, X., Chen, C., Liu, X. (2023). Research advances in the impacts of biochar on the physicochemical properties and microbial communities of saline soils. Sustainability, 15(19), 14439.
63. El-Ghamry, A., El-Sherpiny, M. A., Helmy, A. A. & Kassem, M. A. Sustainable agriculture through soil amendments and foliar nutrition: Mini review of the benefits of biochar, zeolite, compost and micronutrients in mitigating deficit irrigation water. *J. Soil. Sci. Agricultural Eng.*, 255–262. (2024).
64. Qin, X., Wang, Z., Lai, J., Liang, Y. & Qian, K. The synthesis of selenium nanoparticles and their applications in enhancing plant stress resistance: A review. *Nanomaterials* **15** (4), 301 (2025).
65. Zahran, E., Wang, B., Amoah, K., Elbahnaswy, S., Ahmed, F., Farid, O. A. H. A., …Abdel-Hafez, L. J. M. (2025). Dietary selenium nanoparticles (SeNPs) mediate intestinal microbiome andmicrobiota-derived metabolites regulating the growth and immunity of Nile tilapia. *J. World Aquaculture Soc.* 56(2), e70019.
66. Han, Y. et al. Agronomic practices-driven response of nitrogen-related microorganisms. *Plant. Soil.*, 1–16. (2025).
67. Hossain, M., Jotisankasa, A., Aramrak, S., Kamchoom, V., Nishimura, S., Nontananandh,S., Semmad, S. (2025). Increased erosion in biochar-amended soil: importance of integrating erosion control blankets and vegetation. *Biogeotechnics*, 100161.
68. Soliman, M. H., Alnusairi, G. S., Khan, A. A., Alnusaire, T. S., Fakhr, M. A., Abdulmajeed,A. M., Najeeb, U. (2023). Biochar and selenium nanoparticles induce water transporter genes for sustaining carbon assimilation and grain production in salt-stressed wheat.*J. Plant Growth Regulation*, **42** (3), 1522–1543.
69. Zhou, H., Guo, J., Liu, H., Wang, J. & Wang, Y. Effects of Biochar pyrolysis temperature and application rate on saline soil quality and maize yield. *Agronomy* **14** (7), 1529 (2024).
70. Khatoon, Z., Orozco-Mosqueda, M. D. C. & Santoyo, G. Microbial contributions to heavy metal phytoremediation in agricultural soils: A review. *Microorganisms* **12** (10), 1945. (2024).
71. Li, X., Yu, Y., He, R., Zhen, Q. & She, D. Synergistic effects of aged lignin-based Biochar and selenium fertilization on heavy metal remediation in agricultural soils. *Ind. Crops Prod.* **225**, 120464 (2025).
72. Wu, Y. et al. Fertilization of potentially toxic element-contaminated soils remediated with reusable biochar pellets using rice straw, pig manure and their derived Biochar. *Environ. Pollut.* **366**, 125551 (2025).
73. Amalina, F. et al. Advanced techniques in the production of Biochar from lignocellulosic biomass and environmental applications. *Clean. Mater.* **6**, 100137 (2022).
74. Maurice, N. Impact of nanoparticles on soil microbes for enhancing soil fertility and productivity. In *Nanotechnology for Sustainable Agriculture* (75–127). (Apple Academic, 2023).
75. Pokharel, P., Ma, Z. & Chang, S. X. Biochar increases soil microbial biomass with changes in extra-and intracellular enzyme activities: a global meta-analysis. *Biochar* **2**, 65–79 (2020).
76. Zhang, L., Chang, L., Liu, H., de Jesús Puy Alquiza, M. & Li, Y. Biochar application to soils can regulate soil phosphorus availability: a review. *Biochar* **7** (1), 1–20 (2025).
77. Zhao, X. et al. Regulation pathways of Biochar and endophytic bacteria in Na + translocation and salt stress alleviation in the soil-plant system. *Plant. Growth Regul.*, 1–13. (2025).
78. Shi, W., Zhou, J., Li, J., Ma, C., Zhang, Y., Deng, S., Luo, Z. B. (2021). Lead exposure-induced defense responses result in low lead translocation from the roots to aerial tissues of two contrasting poplar species. *Environ. Pollut.*, **271**,116346.
79. Arinzechi, C. et al. Synergistic mitigation of cadmium stress in rice (Oryza sativa L.) through combined selenium, calcium, and magnesium supplementation. *Environ. Geochem. Health*. **46** (11), 435 (2024).
80. Golubkina, N. et al. Effect of foliar application of sodium selenate on mineral relationships in Brassicaceae crops. *Horticulturae* **9** (5), 535 (2023).
81. Shahbaz, M., Akram, A., Mehak, A., Haq, E. U., Fatima, N., Wareen, G., Sabullah,M. K. (2023). Evaluation of selenium nanoparticles in inducing disease resistance against spot blotch disease and promoting growth in wheat under biotic stress. *Plants* **12** (4), 761.
82. Saffan, M. M. et al. Sustainable production of tomato plants (Solanum lycopersicum L.) under low-quality irrigation water as affected by bio-nanofertilizers of selenium and copper. *Sustainability* **14** (6), 3236 (2022).
83. Pal, P. & Malhotra, M. Emerging technologies for selenium separation and recovery from aqueous systems: A review for sustainable management strategy. *Can. J. Chem. Eng.* **101** (5), 2859–2877 (2023).
84. Di, X. et al. Regulatory effects and mechanism of different selenium species on cadmium accumulation in Triticum aestivum L. (microbial response, gene expression and element accumulation). *Environ. Res.* **264**, 120374 (2025).

85. Batista, A. et al. Application of silver and selenium nanoparticles to enhance plant-defense response against biotic stressors. *Acta Physiol. Plant.* **47** (2), 21 (2025).
86. Sakthivel, A., Chandrasekaran, R., Balasubramaniam, S., Sathyanarayanan, H. & Gnanajothi, K. Nanomaterials as potential plant growth modulators: applications, mechanism of uptake, and toxicity. *Compr. Rev. BioNanoScience*. **15** (1), 1–21 (2025).
87. Sodhi, G. K., Wijesekara, T., Kumawat, K. C., Adhikari, P., Joshi, K., Singh, S., Mitra, D. (2025). Nanomaterials-plants-microbes interaction: plant growth promotion and stress mitigation. *Front. Microbiol.*, **15**, 1516794.
88. Ochoa, L., Shrivastava, M., Srivastava, S., Cota-Ruiz, K., Zhao, L., White, J. C., Gardea-Torresdey, J. L. (2025). Nanomaterials for managing abiotic and biotic stress in the soil–plant system for sustainable agriculture. Environmental Science: Nano.
89. Kumar, A., Jain, G., Dutta, P., Singh, P., Alam, N., Narayan, S., Chakrabarty,D. (2024). Nanopriming with phytofabricated selenium nanoparticles alleviates arsenite-induced oxidative stress in *Spinacia oleracea* L. *Environ. Sci. Pollut. Res* 1–17.
90. Maqbool, S., Ramzan, T., Haider, A., Waraich, E. A., Fatima, A., Siddiqui, M. H. ur Rehman, H. (2025). Impact of Thiourea on Wheat's Morpho-Physiological and Ionic Attributes (*Triticum aestivum* L.) under Lead Stress: Reducing the Translocation of Lead from Soil to Roots, Shoots,and Grains. ACS Omega.
91. Murtaza, G. et al. Biochar-Soil-Plant interactions: A cross talk for sustainable agriculture under changing climate. *Front. Environ. Sci.* **11**, 1059449 (2023).
92. Bano, A., Hassan, T. U., Waqar, A., Mushtaq, T. & Urooj, N. Impact of Biochar on climate change, agricultural soil and plants. *Commun. Soil Sci. Plant Anal.* **56** (3), 419–435 (2025).
93. Singh, S., Khan, N. A., Shehata, N., Singh, J. & Ramamurthy, P. C. Insight into Biochar as sustainable biomass: production methods, characteristics, and environmental remediation. *J. Clean. Prod.*, 143645. (2024).
94. Farouk, S., Al-Huqail, A. A. & El-Gamal, S. M. Potential role of Biochar and silicon in improving physio-biochemical and yield characteristics of borage plants under different irrigation regimes. *Plants* **12** (8), 1605 (2023).
95. Moulick, D., Mukherjee, A., Mahanta, S., Chowdhara, B., Pattnaik, B. K., Mukhopadhyay,A. K., Hazra, S. (2025). Nano-composites and nano-biochar: a novel solution to alleviate heavy metal stress in crops. In: *Nanomaterials and Nano-Biochar in Reducing Soil Stress* (183–224). Apple Academic Press.
96. Younas, H., Bareen, F. E., Nazir, A. & Shafiq, M. Appraisal of bioavailability and immobilization of constituent metals in tannery solid waste Biochar in soil amendments using sunflower. *Soil. Sediment. Contamination: Int. J.* **34** (2), 153–169 (2025).
97. Amubieya, O. F., Akanbi-Gada, M. A. & Ogunkunle, C. O. Influence of Biochar on Lead–Induced oxidative damage and Anti-Oxidative defense mechanisms on the leaf of *Solanum lycopersicum* L. (Tomato). *Jewel J. Sci. Res.* **9** (1&2), 45–58 (2024).
98. Anwar, T. et al. Alleviation of cadmium and drought stress in wheat by improving growth and chlorophyll contents amended with GA3 enriched Deashed Biochar. *Sci. Rep.* **13** (1), 18503 (2023).
99. Ali, A., Ali, N., Gu, Y., Ahmad, N., Ali, N., Ahmad, I., Khan, M. I. U. (2025).Application of fertilizers improves cadmium phytoextraction efficiency of *Nasturtium officinale* R. Br. grown in cadmium-contaminated water. *Environ. Monitoring Assessment* **197** (2), 192.
100. Seehausen, M. L., Gale, N. V., Dranga, S., Hudson, V., Liu, N., Michener, J.,Thomas,S. C. (2017). Is there a positive synergistic effect of biochar and compost soil amendments on plant growth and physiological performance? *Agronomy* **7**(1), 13.
101. Liang, J., Li, Y., Si, B., Wang, Y., Chen, X., Wang, X., Biswas, A. (2021). Optimizing biochar application to improve soil physical and hydraulic properties in saline-alkali soils. *Sci. Total Environ.*, 771, 144802.
102. Murtaza, G., Usman, M., Iqbal, J. et al. The impact of biochar addition on morpho-physiological characteristics, yield and water use efficiency of tomato plants under drought and salinity stress. BMC Plant Biol 24, 356 (2024).
103. Cao, Y., Turk, K., Bibi, N., Ghafoor, A., Ahmed, N., Azmat, M., Ahanger, M. A.(2025). Nanoparticles as catalysts of agricultural revolution: enhancing crop tolerance to abiotic stress: a review. *Front. Plant Sci.*, **15**, 1510482.
104. Peng, X., Liu, H., Chen, P., Tang, F., Hu, Y., Wang, F., Shen, S. (2019). A chromosome-scale genome assembly of paper mulberry (Broussonetia papyrifera) provides new insights into its forage and papermaking usage. *Molecular Plant* **12**(5), 661–677.

Acknowledgements
The authors acknowledge and appreciate the Ongoing Research Funding Program (ORF-2025-801), King Saud University, Riyadh, Saudi Arabia.

Author contributions
G.M., N.E.H., M.U., Q.Z., M.R., G.D., Z.A., A.M., J.I., and M.S.E., designed and conceived the study idea. G.M., completed the experiments. M.S.E., N.A., S.A., L.G., R.I., U.M.-T., M.G., analysed the data and performed visualizations and statistical data analysis. G.M., M.R., wrote the original draft. M.R., Q.Z., G.D., Z.A., J.I., M.S.E, and M.R., reviewed and edited the manuscript. R.I., and M.S.E., reviewed the manuscript and provided funds. M.S.E., M.R., and N.A., provided the resources and supervision. All authors made valuable revisions and edited the manuscript and approved the last version.

Funding
This research was supported by the Ongoing Research Funding Program (ORF-2025-801) at King Saud University in Riyadh, Saudi Arabia.

Declarations

Competing interests
The authors declare no competing interests.

Permission
Permissions or licenses were obtained to collect Barley seeds (Hordeum vulgare JAU-83) taken from (Regional Agricultural Research Institute Bahawalpur) and waste of *Broussonetia papyrifera* from from Lal Suhanra Park before starting the research.

Statement on guidelines
All experimental studies and experimental materials involved in this research. are in full compliance with relevant institutional, national and international. guidelines and legislation.

Additional information

Correspondence and requests for materials should be addressed to G.D., Z.A., R.I. or M.G.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

© The Author(s) 2025