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Mitigation of arsenite toxicity in barley using iron-biochar nanocomposites and *Bacillus* sp.: impacts on soil immobilization, plant stress, and health risk

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Abstract

Gradual rise in arsenic (As) pollution due to reuse of unprocessed industrial wastewater for cultivation of crops is causing lethal effects on human health. Moreover, consumption of As-contaminated grains as a food is a global concern. Hence, effective alternatives to manage this problem are urgently needed. This research studied the combined role of iron-biochar nanocomposites (Fe-BCNC) and plant growth promoting (PGPR) *Bacillus* sp. in arsenite [As (III)] detoxification in soil. The investigation focused on the growth of barley, *Hordeum vulgare* (*H. vulgare*), in an As (III)-contaminated soil and assesses As-associated health risks. Specifically, the study highlighted the impact of various treatment combinations of Fe-BCNC and PGPR with respect to As fractions in soil, As translocation, bioaccumulation, grain quality attributes, stress markers (like antioxidant enzymes) and health risks associated with As (III)-contaminated food intake. The Fe-BCNC was added into the soil at the rate of 80 g kg^{-1} of soil before sowing. The results revealed that As applied at 25 and 50 mg kg^{-1} enhanced phytotoxicity, causing reduced growth, and worsening physiological and biochemical attributes of *H. vulgare*. Fe-BCNC and PGPR promoted reductions in As toxicity in both soils (25 and 50 mg kg^{-1} As) by improving plant height (26, 52%), grain weight (75, 76%), physiological such as electrolyte leakage (37, 46%) and grain quality attributes such as crude protein (49, 59%) further regulated by antioxidant enzymes (CAT 31, 26; SOD 31, 25%; POD 48, 30%) under stress conditions. The combined treatment of Fe-BCNC with PGPR enhanced As immobilization in soil (40, 47%), respectively, with the highest proportion of iron, aluminum and manganese associated to As. Moreover, the same combination resulted in reduced As accumulation and translocation, lowering health risk (92, 94%) and cancer index (2×10^{-4}, 2×10^{-3}) in both soils, respectively. Formulations of Fe-BCNC and PGPR and their combination as biodynamic amendment could be crucial to alleviate As-related issues and prevent health risks in end consumers. This research helps achieving Sustainable Development Goals 2 (zero hunger) and 15 (life on land) by ameliorating food production, remediating tinted soil, and reconstituting the environment.

Keywords Abiotic stress, Arsenic fractionation, Health risk, Microorganisms, Nanotechnology phytotoxicity

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Introduction

Environmental pollution due to arsenic (As) is a worldwide ecological, agronomic, and health threat. Geothermal waters, volcanic eruptions, pesticides, fertilizers and industrial waste are reported as a major source of As contamination [22], being ranked as a first place concern among all metal(loid)s by Agency for Toxic Substances and Disease Registry (ATSDR), US, especially due to its carcinogenic nature [9, 136, 50, 97]). Arsenic is found in soil and subsoil surfaces in Pakistan, with deeper soils having evolved content than surface soil in the same area. Sindh's agricultural areas had the highest soil As content (46.10 mg kg^{-1}), followed by 36.01 mg As kg^{-1} in soil from various locations of Punjab. Arsenic consumption from food was 343.5 g/day in arsenic-endemic districts [92]. Even at extremely low concentrations, As has shown drastic effects on plants, affecting different morphological, physiological, and biochemical parameters [2]. Ethylene production, membrane damage, reduced transpiration efficiency, enhanced oxidative stress, as well as drops efficiency of the protein synthesis systems, are common symptoms shown by plants such as *H. vulgare* under As stress, thus hindering plant growth [19].

The remediation of As-polluted soils, or the use of transformation procedures to decrease their bioavailability in plants, have gained increased attention. Procedures for soil remediation include physical methods (excavation of soil), chemical methods (solvent extraction, ion exchange, oxidation, reduction), and biological methods (biodegradation, biosorption, biotransformation and phytoremediation) [51, 83]. Physical and chemical remediation methods are highly effective and useful, but their restricted utilization is mostly due to their arduous, time and energy-intensive nature as well as to generation of hazardous materials in large quantities [78, 101]. Regarding As removal, adsorption is considered among the most effective methods, being quick, easy to handle, and ecologically friendly [134].

Currently, nanotechnology is being proposed as a next-generation environmental remediation tool to give effective solutions for a variety of complex environmental issues due to its high adsorption capacity. Nanocomposites frequently have shown remarkable improvements in numerous features such as pore characteristics, functional groups, and therefore adsorption sites; also, separation becomes simple and quick [135]. Anaerobic pyrolysis produces biochar from a variety of biomass sources, including agricultural and forestry leftovers. Within this field, magnetic iron oxide nanoparticles have long been employed as in situ arsenic cleanup agents in contaminated soils, which is due to its unique affinity for arsenic over other metals [121], enormous specific surface area [75], and flexibility of extraction from aqueous solution [12]. Metal oxide nanocomposites based on biochar are a novel class of modified biochar substances that combine the benefits of biochar and nanomaterials [65, 120]. Metal biochar nanocomposites limit metal mobility in contaminated soils by ion exchange, adsorption, precipitation, and biochar–metal complex formation [51, 106]. The combination of the high surface area and catalytic properties of iron nanoparticles with the structural integrity and stability of biochar leads to a synergistic effect, resulting in enhanced overall performance [114]. The use of iron-modified biochar for arsenic removal from water/soil have be studied intensively [125, 127, 128, 130]

Another very interesting field is related to detoxification and transformation of metals by using PGP microbes, which has been previously reported in specific literature [59, 80, 82] as they enhances plant growth in metal contaminated soil by reducing metal accumulation in edible parts. As-oxidizing bacteria in combination with chemical treatments can immobilize As from polluted environments [15]. Precipitation, biosorption, and enzymatic transformation are methods that microbes utilize to convert heavy metals into less hazardous forms, which are less mobile, more stable, or inactive [59]. The buildup of HMs in food crops such as barley (*Hordeum vulgare*) is alarming because HMs cause health problems in humans, economic losses, and deterioration of food quality [137]. During 2023–2024, the cultivation of Barley in Pakistan has escalated to 6.8 percent to 42 thousand tonnes, compared to last year [34, 138]. Barley grains are rich in starch, vitamin E, B6, magnesium, and iron (with antioxidant properties) [37].

Despite the fact that plant–bacteria interactions play a crucial role in plant development and metal(loid)s availability and toxicity, most of the disseminated research is focuses on the independent effect of microbial communities and nanoparticles on As transformation and bioaccumulation. Although the sole application of iron-modified biochar and bacteria has been used for a long time for the remediation/treatment of HMs contaminated drinking or waste water (Kolodynska et al., [57, 139, 44, 123]), there is not enough information available about the use of iron-doped biochar nanocomposites along with growth promoting bacteria as a co-benefiter for soil As (III) reclamation while simultaneously improving crop growth and development [30, 141, 85]. Furthermore, little is known about how crops react physiologically or biochemically to Fe-BCNC treatment in As-contaminated soils. Moreover, limited studies have examined the transformation of As fractions in soil and associated reductions in grain As content and food-chain risk under combined nano-bio interventions. Investigating the effects of combining Fe-BCNC and metal-tolerant bacteria on As uptake, plant

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growth, and physiological and biochemical reactions in crops cultivated in As-contaminated soils is clearly needed to increase knowledge in this field. In this regard, for the current study we hypothesized that the application of combined nanocomposites and PGPR could be a more effective solution for enhancing growth and the physiological processes of plants by reducing the harmful effect of arsenic. In view of that, the objectives of this research were (1) to evaluate the effects of individual as well as combined usage of PGPR and nanocomposites on the growth, physiological functions, and biochemical characteristics of *H. vulgare* cultivated in As-contaminated soil; (2) to assess the effectiveness of nanocomposites and bacteria in the phyto-stabilization of As-polluted soil, also performing a health risk assessment associated to the consumption of *H. vulgare* cultivated in As-stressed conditions.

Materials and methods

Preparation of iron-biochar nanocomposites

Wheat straw biochar was produced using the method described by Haider et al. [42], while iron-biochar nanocomposites (Fe-BCNC) were prepared following the method described by Priyadarshni et al. [91], with biochar being provided by the Soil and Environmental Microbiology Laboratory, UAF (Pakistan). Briefly, an amount of 400 g of biochar was added in a 2 M NaOH solution and allowed to shake for 24 h on a mechanical shaker. Later on, the filtered and washed biochar was oven-dried at 50 °C for 4 h. This biochar was dipped in 1 L of a 1 M $FeCl_3$ solution and kept at room temperature for 24 h. Then, 0.5 M NaOH was slowly incorporated in the solution (with continuous stirring) to achieve the precipitation of iron oxide on the surface of the biochar. The solution was kept around 30 min to allow the reaction to take place, and, afterwards, the Fe-BCNC composites were washed several times, dried, collected and stored in an airtight container at 28 ± 2 °C in the lab for characterization and further use.

Characterization of nano-biocomposites

The prepared composites were characterized for Fourier transform infrared spectroscopy (FTIR) (Tensor 27 FTIR, Bruker Optics GmbH, Germany). The pressed pellet of mixture of biochar and potassium bromide (KBr) was scanned multiple times to obtain IR spectra. The measurements were done in mid-infrared region of 600–4000 cm^{-1} [53]. Copper alpha *X*-ray analysis was used for the detection of crystalline structures using *X*-ray diffraction (XRD) (D8 Advance, Bruker, Germany), while Scanning Electron Microscopy (SEM) (cube II, Emcraft, South Korea) was also performed to provide images additionally

helping in the assessment of the structure of the prepared composites.

Bacterial characterization, inoculation and screening for metal resistance

The inoculum of pre-isolated plant growth promoting bacterial strain *Bacillus* sp. MN-54 (Accession No. KT-375574) obtained from the Soil and Environmental Microbiology Laboratory, UAF (Pakistan) was prepared in tryptic soya broth (TSB) [68]. The minimum inhibitory concentration (MIC) test was performed in triplicates, before seed inoculation to check the bacterial ability to tolerate as stress [109]. The test was conducted in tryptic soya agar (TSA) in which arsenic trioxide (As_2O_3) was used as a source of arsenic contamination in controlled laboratory conditions. The plates were incubated at 28 ± 2 °C for 42 h with the bacterial strain showing resistance toward arsenic up to 150 mg As kg^{-1}. The considerable growth of the bacteria in the presence of As over 42 h at 28 °C was esteemed as a demonstration of As tolerance. The method described by Shagol et al. [111] was used to test the ability of microbes to convert As (III) into As (V). Plates were incubated at 28 ± 2 °C for 3 days, and then a volume of 10 mL of 0.1 M $AgNO_3$ was added. The appearance of a brown precipitate on As (III) plates indicates As (III) oxidation. The bacterial inoculum of strain MN-54 was prepared in sterilized TSB by placing it on a shaker for one day. The culture was collected after a 20 min centrifugation at 6000 rpm to obtain cell density up to 10^8–10^9 CFU mL^{-1} [104, 133].

Plant material

The seeds of the *H. vulgare* variety "Talbina 2021" were provided by the Ayyub Agricultural Research Institute (AARI), Faisalabad (Pakistan). Surface sterilization was done following Naseem et al. [82]. Following Abd-Alla et al. [3],surface disinfected seeds of *H. vulgare* were inoculated with strain MN-54 in the mixture of broth containing sugar, peat and clay having 1:1 (*w/w*) ratio of peat and clay [17]. As control treatment, sterilized peat plus clay mixture, having sugar solution with broth without bacterium, was used.

Experimental design and treatments description

Pot experiments were performed as follows. Eight kilograms of soil samples (both non-contaminated and contaminated samples) were placed into 10 kg plastic pots with dimensions 30 cm length × 25 cm wide, as per the treatment plan. The experimental treatments were divided into 12 groups: T1: control [positive control], T2: Fe-BCNC; T3: As 25 mg kg^{-1} [negative control]; T4: As 50 mg/kg [negative control]; T5: PGPR; T6: PGPR + Fe-BCNC; T7: PGPR + As 25 mg/kg; T8: PGPR + As 50 mg/

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kg; T9: Fe-BCNC + As 25 mg/kg; T10: Fe-BCNC + As 50 mg/kg; T11: Fe-BCNC + PGPR + As 25 mg/kg; T12: Fe-BCNC + PGPR + As 50 mg/kg. Arsenic trioxide (As_2O_3) was used as a source of As (III) contamination with the rate of 0, 25 and 50 mg kg^{-1} added to the soil samples before sowing *H. vulgare* seeds. The prepared nano-bio-composites were added at the rate of 80 g kg^{-1} of soil before sowing. The experiment was carried out using a completely randomized design (CRD), with three replicates per treatment. Table **S1** and **S2** (Supplementary data) shows the characteristics of soil used in this study and some environmental conditions of studied area.

Estimation of agronomic, physiological and grain quality attributes

Agronomic parameters were measured after 112 days of sowing and preserved after harvesting. The plant height and root length was taken with the help of a measuring tap while the fresh weight was taken with electrical balance. The *H. vulgare* plant samples were kept in oven at 70 °C and dry mass was determined. Quantification of relative water content (RWC) of plants leaves was carried out by using the method indicated in Akhtar et al. [6]. The chlorophyll contents were analyzed as per Arnon, [14]. The standard method included in Gong et al. [41] was followed for measuring electrolyte leakage (EL).

Amounts of 0.5 g of homogenized and oven-dried powdered plant samples (root, shoot and grains) were digested using the di-acid mixture method, adding H_2SO_4 and HNO_3 at a 9:4 rate [36]. By following the method of Ryan et al. [95], phosphorus (P) in soil, plant root, and shoot were investigated through spectrophotometer (T60UV, Leicester, UK). The method of Chapman and Pratt [26] was used for the quantification of total nitrogen contents in plant root, shoot and gains. The grain quality attributes such as ash, crude protein, crude fiber and crude fat of grains were analyzed according to the methods included in AOAC [10].

Antioxidant enzymes

Fresh leaves of *H. vulgare* (at 40 days) were ground with potassium phosphate buffer and centrifuged for 18 min at 10,000 rpm for 15 min to collect sample extracts.

For the determination of superoxide dismutase (SOD), plant samples were thoroughly mixed with ethylene diamine tetra acetic acid (EDTA), nitro blue tetrazolium (NBT), riboflavin, and methionine, and absorbance was measured by means of a spectrophotometer (T60UV, UK) [69]. Catalase enzymatic activity was assessed based on the Chance and Maehly [25] technique: H_2O_2 cell extracts were added in a mixture of phosphate buffer, and absorbance was measured at the designated wavelength. The protocol by Hemeda and Klein [45] was used to evaluate peroxidase activity (POD). The reaction mixture for POD analysis comprised guaiacol, phosphate buffer, H_2O_2, and the extract, with absorbance being measured at 470 nm.

Arsenic fractionation in soil and bioaccumulation in plant parts

Arsenic fractionation was carried out as per Motuzova et al. [77], with details included in Table 1. Arsenic fractions were classified as mobile (specifically and nonspecifically adsorbed arsenic ions), tightly bound (low-solubility As and As in primary and secondary minerals), and As compounds connected with Fe, Al, and Mn oxides and organic molecules (Table 1). Atomic absorption spectrophotometry (Hitachi Polarized Zeeman AAS, Z-8200, Japan) was used to evaluate the As content in soil samples as well as in plant extracts.

Table 1 Steps corresponding to the sequential fractionation of arsenic in soil samples

Reagents used	Mechanical treatment	Fraction
1% $(NH_4)_2SO_4$ + 0.25% $(NH_4)_2MoO_4$, pH 5.5–6.0	Sample: solution at 1:50, shaking for 4 h, and 10 min centrifugation at 4000 rpm	Nonspecifically adsorbed (exchangeable ions)
0.5 M $Na_3C_6H_5O_7$ + 1 M $NaHCO_3$ + 0.13 M $Na_2S_2O_4.2H_2O$	Sample: solution at 1:50, water bath for 15 min (at 85 °C) and 10 min centrifugation at 4000 rpm	Compounds bound with Fe, Mn and Al oxides
0.1 M NaOH	Soil: solution at 1:50, 2 h shaking, 20 h extracting, 10 min centrifuging at 4000 rpm	Compounds bound with organic substances
1 M HNO_3	Soil: solution at 1:50, 1 h shaking, 10 min centrifuging at 4000 rpm	Compounds bound to carbonates and low-solubility salts
Melting with NaOH	Evaporation on a water bath (at 85 °C), solution of residue in 1 M HNO_3	Residual arsenic

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Estimation of translocation and health risk

Translocation, bioaccumulation factors and bio-concentration factors (TF, BAF and BCF, respectively) were calculated by using Eq. (1), (2) and (3) [112]:

$$TF = \frac{Cshoot}{Croot}, \tag{1}$$

$$BAF = \frac{Cplant}{Csoil}, \tag{2}$$

$$BAF = \frac{Croot}{Csoil}, \tag{3}$$

where C is the As concentration in each of the different substrates (shoot, root, plant, and soil).

The average daily intake [ADI] (mg kg^{-1} day^{-1}) of *H. vulgare* was estimated by using Eq. (4) [112]:

$$ADI = \frac{CAs \times IR}{BW}, \tag{4}$$

where CAs is the As concentration in grain, IR represents the ingestion rate of *H. vulgare* (60 g day^{-1} person^{-1}) and BW is the average body weight (70 kg).

Integrated lifetime cancer risk (ILTCR) in As-contaminated *H. vulgare* grain ingestion was computed using Eq. (5) [96]:

$$ILTCR = ADI \times CSF, \tag{5}$$

where CSF represents the cancer slope factor for As (1.5 mg kg^{-1} day^{-1}).

Statistical analysis

The results were analyzed under CRD through three-way factorial ANOVA using SPSS software (SPSS. 26.0, IBM, USA). Differences among treatments was compared using Duncan multiple range test having 3 replications of each treatment, with the level of statistical significance being fixed at $P \leq 0.05$. Some of the ANOVA tables are given in supplementary data file. The software Origin Pro 2021 (64-bit: 9.8.0.200) (Northampton, Massachusetts, USA) was used for generation of figures (Figs. 2, 3, 4, 5 and 6). The R programing (version 4.3.1) was used for the generation of PCA biplot (Figure S1) and Fig. 7 was created using GraphPad prism (8.0) (GraphPad Software, San Diego, CA, USA).

Results

Iron biochar nanocomposites characterization

The surface morphology and microstructure of the prepared nano-bio-composites was examined by means of SEM, with results shown in Fig. 1a, b. When the image was amplified 2158 times biochar depicted a rough surface with thread-like structure (Fig. 1a). However, it also exhibited a needle-like structure with smooth surface. A high level of porosity was also observed and deposition of iron particles in spherical shape, on the surface of biochar was clearly visible when the sample was amplified 6700 times (Fig. 1b).

Nanocomposites were examined through a KBr disc, providing results of a range of frequencies that fall over the functional groups regions and fingerprint reigns according to functionalities in the samples (Fig. 1c). Two characteristic peaks at frequency (*v,* expressed in cm^{-1}) 2931 and 2810 confirm anti-symmetric and symmetric (respectively) stretch of methyl group (-CH$_3$), whereas other prominent peaks at 2366 and 1740 reveal the carbonyl stretch (C=O). Bending vibrations (expressed in cm^{-1}), 694 and 627 at the last region were observed, which confirmed metal interaction with groups attached with structure. The spectra also showed that there was no characteristic peak in the range of 3000–3500 cm^{-1}, which indicates absence of the OH group. The distinctive vibrations of C–O bands (at 1217 cm^{-1}) confirm the existence of these groups, which may have been produced as a result of SiO2 hydrolytic cleavage from the polycyclic framework. The shift of the band from 1700 to 1709 cm^{-1} indicates the presence of covalent connections between biochar and the metal oxide nanoparticles in the final composite. The peaks that emerged in the range of 400–850 cm^{-1} correspond to the iron–oxygen linkage (Fe–O).

The crystalline structure of biochar was determined by means of XRD (Fig. 1d), showing sharp peaks which confirm the crystalline nature of biochar coated with iron nano-particles, and also the presence of SiO$_2$ and Al/Si oxides in biochar, with the typical results of 21.23°, 21.30°, 22.62°, 23.23°, 29.99°, 26.53°, 28.49°, and 39.41° in the 2θ degree with respect to intensities 46, 56, 53, 55, 45, 58, 49 and 35. XRD patterns for fabricated biochar materials highlight the crystal planes 31, 36, 22, 36, 30, 40, and 32 according to 2θ values of 30.20°, 35.64°, 36.92°, 42.92°, 52.85°, 57.41°, and 62.91°, respectively, compared with the standard, which indicates that the biochar materials were successfully modified and finally coated with Fe$_3$O$_4$ nanoparticles.

Agronomic attributes

Table S3, Supplementary Material shows the effect of Fe-BCNC and PGPR on the growth of H. vulgare, both in unpolluted and As-contaminated soils. Plant growth (height, root length, shoot dry biomass, and root dry biomass) were significantly reduced ($P \leq 0.05$) in As-polluted soil samples compared to uncontaminated soil. The use of Fe-BCNC and PGPR (each one alone, as well
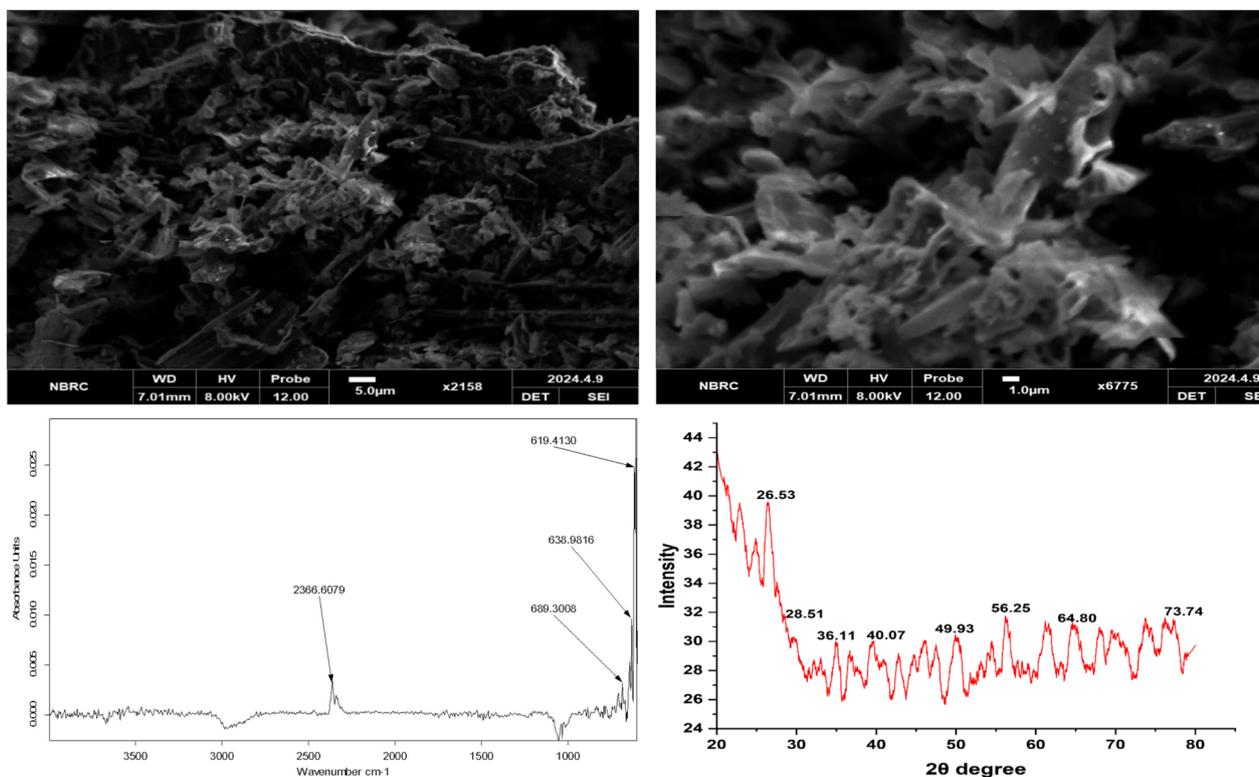
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Figure. 1. Characterization of Fe-BCNC. SEM images of Fe-BCNC at 2000 and 6700 amplifications (**A** and **B**), FTIR spectra (**C**), and XRD results (**D**) for the iron nano-bio-composites studied

as in combination) resulted in improvement of As stress-induced growth reduction of H. vulgare. The combined application of PGPR + Fe-BCNC resulted in improved plant development, surpassing that of the control group. Fe-BCNC and PGPR showed considerable (and statistically significant, at $P \leq 0.05$) improvement in growth under normal (unpolluted) soil. The application of Fe-BCNC + PGPR on soil samples that were polluted with As at doses of 25 and 50 mg kg^{-1} caused an improvement in plant height by 26% and 52%, root length by 74% and 91%, shoot dry biomass by 29% and 26%, root dry biomass by 85% and 122%, and leaf area by 74% and 72%, respectively. Fe-BCNC and PGPR enhanced growth in As-contaminated soil samples, resulting in percentage increases in several growth properties compared to normal (unpolluted) soil samples.

Physiological attributes

The As stress both at 25 and 50 mg kg^{-1} significantly reduced chlorophyll contents and relative water contents (Table S4, Supplementary Material), however, the combined application of Fe-BCNC and PGPR improved relative water content up to 28% and 30% under 25 and 50 mg As kg^{-1} stress conditions, respectively. The highest increase in chlorophyll and carotenoids was observed for

the combined application of Fe-BCNC + PGPR, reaching up to 15% and 28%, and 47% and 100%, for 25 and 50 mg As kg^{-1}, respectively. In addition, arsenic stress increased the electrolyte leakage (EL) in plant leaves as compared to the control. The highest EL was observed under the 50 mg As kg^{-1} dose of the pollutant, which is around 110% above the levels in the control. However, the combined application of Fe-BCNC + PGPR reduced EL up to 37% and 46% for the pollutant doses of 25 and 50 mg As kg^{-1}, respectively, as compared to the respective controls.

Chemical attributes

The results revealed that the As stress (both at 25 and 50 mg kg^{-1}) significantly reduced the values of different chemical parameters such as N and P contents of *H. vulgare* as well as in soil samples. The application of Fe-BCNC + PGPR resulted in statistically significant differences in comparison to the respective controls (Fig. 2a-e). The results depicted that the combined addition of Fe-BCNC and PGPR in soils polluted with both As doses (25 and 50 mg kg^{-1}) significantly increased plant N contents in shoots (19% and 16%) and grains (46% and 41%), as well as P contents in shoots (33% and 50%) and grains (71% and 72%), as compared to the respective controls.
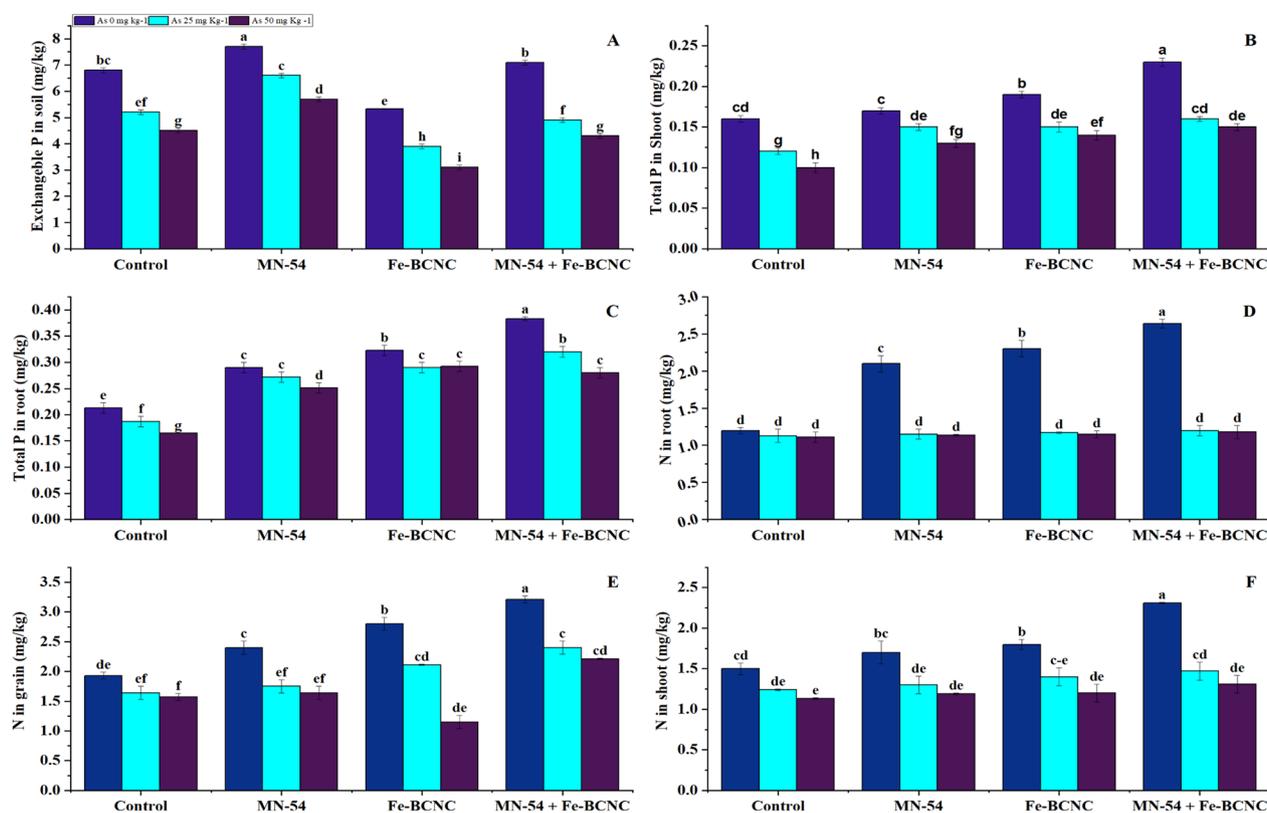
Figure. 2. Effect of Fe-BCNC and PGPR on P and N contents of *H. vulgare* (root, shoot, grain) under arsenic stress, with different doses of As indicated using different colors for histograms. Columns represent mean values (*n* = 3) whereas bars are indicating the standard error (SE). Different letters indicate significant difference among all mean values according to Duncan multiple range test under CRD at P ≤ 0.05. MN-54: plant growth promoting bacterial strain *Bacillus* sp.; Fe-BCNC: iron-biochar nanocomposite; MN-54 + Fe-BCNC: plant growth promoting bacterial strain *Bacillus sp.* and iron-biochar nanocomposites

Grain quality analysis

The results revealed that the arsenic stress (at doses of 25 and 50 mg kg^{-1} As), abates the levels of crude protein (by 49% and 59%), crude fiber (36% and 60%) and crude fat (47% and 55%) of *H. vulgare*. However, the individual and combined application of PGPR and Fe-BCNC significantly (P ≤ 0.05) improved the seed quality of *H. vulgare* in unpolluted as well as in As (III) tainted soil samples (Fig. 3a–d). The combined application of Fe-BCNC + PGPR resulted in an increase in crude fat (75%, 69%), crude fiber (53%, 106%), crude protein (54%, 77%) and ash contents (105%, 88%) in 25 and 50 mg kg^{-1} As polluted soil samples, as compared to the respective controls.

Antioxidants enzymatic activities

The results revealed the existence of significant differences among the treatments for all the antioxidant enzymatic activities (Fig. 3e–g). It was observed that As pollution (both at 25 and 50 mg kg^{-1}) enhanced the antioxidant activity of SOD (by 59% and 112%), POD (129%,

242%) and CAT (50%, 125%), respectively. The highest antioxidant activities were associated with the pollutant addition of 50 mg kg^{-1} As. However, the application of Fe-BCNC + PGPR significantly reduced the enzymatic antioxidant attributes of *H. vulgare*, both for the contaminated and control soil samples. The combined application of Fe-BCNC + PGPR decreased the POD activity in *H. vulgare* leaves by 30% compared to the control (for the pollutant dose of 50 mg kg^{-1} As). Likewise, SOD and CAT activities were significantly reduced in *H. vulgare* by the incorporation of Fe-BCNC + PGPR to the soil samples. The lowest SOD (23 U g^{-1} FW) and CAT (7.1 nmol min^{-1} mg^{-1}) activities were observed under the combined application of Fe-BCNC and PGPR, being 35% and 39% lower in comparison with the control.

Arsenic bioaccumulation in plant parts

At harvest, As was not detected in soil samples, roots, or shoots of *H. vulgare* under unpolluted soil, while 7.1, 1.54, and 0.1 mg As kg^{-1} were found in the roots, shoots and grains, respectively, when 25 mg As kg^{-1} were added

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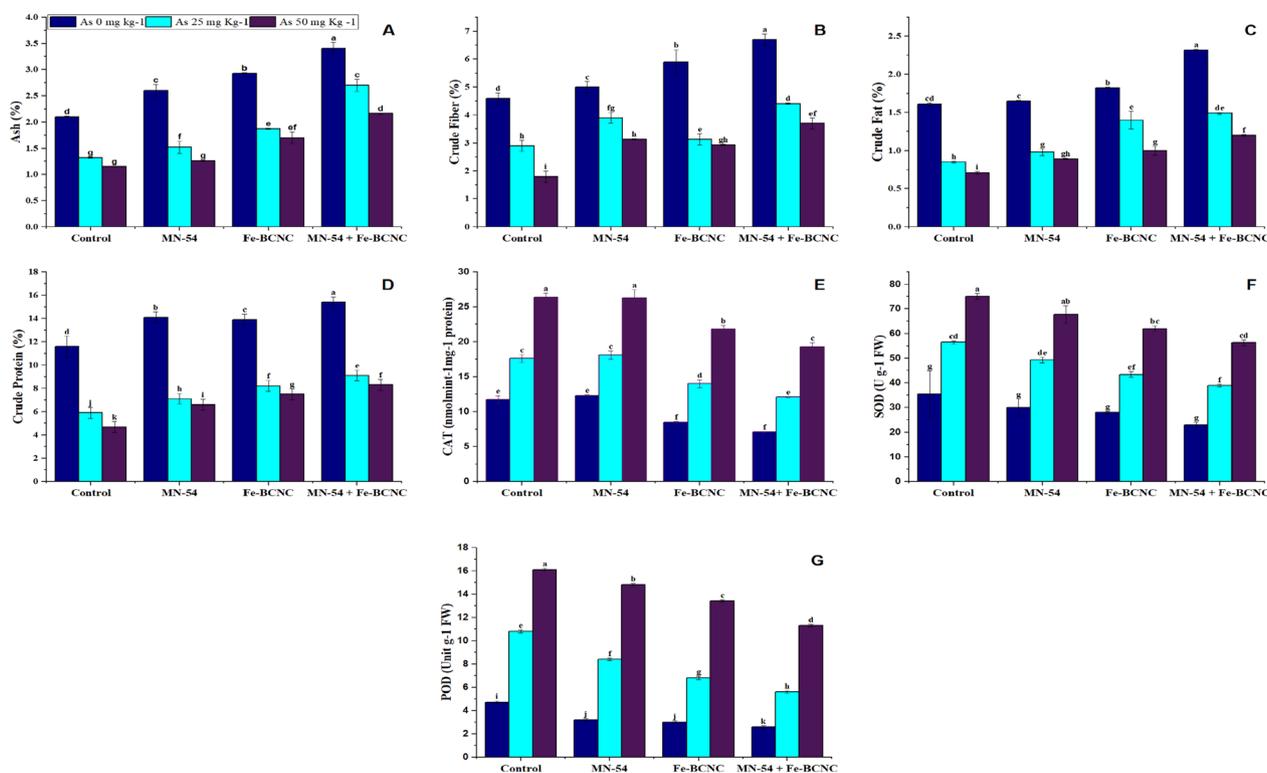
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Figure. 3. Effect of Fe-BCNC and PGPR on proximate composition of *H. vulgare* grains under arsenic stress (**A–D**), with different doses of As indicated using different colors for histograms. Graphs **E–G** represent the antioxidants composition of *H. vulgare* leaves under arsenic stress. Columns are representing mean values ($n = 3$) whereas bars are indicating the standard error (S.E). Different letters are representing the significant difference among all mean values according to Duncan multiple range test under CRD at P ≤ 0.05. MN-54: plant growth promoting bacterial strain *Bacillus* sp.; Fe-BCNC: iron-biochar nanocomposite; MN-54 + Fe-BCNC: plant growth promoting bacterial strain *Bacillus sp*. and iron-biochar nanocomposites; CAT catalase, SOD superoxide dismutase, POD peroxidase

to soil samples, and with these scores reaching 16.90, 3.30, and 1.50 mg As kg^{-1}, respectively, for the pollutant dose of 50 mg As kg^{-1} added to soil (Fig. 4a–c). With the application of Fe-BCNC and PGPR, As contents were significantly decreased in grain, shoot and root samples of *H. vulgare*. Fe-BCNC and PGPR application in the presence of As stress reduced shoot and root As concentrations about 67 and 51 folds in soil samples receiving the 25 mg As kg^{-1} dose, and 72+ and 43-fold in soil samples polluted at 50 mg As kg^{-1}, compared to the respective controls.

Arsenic fractionation in soil

The results revealed that arsenic stress significantly increased the average values of the sum of all the non-specific As-bound compounds in the studied soil samples, as well as other As fractions. Figure 5 shows that the application of Fe-BCNC + PGPR increased the exchangeable As concentration in the soil, with As associated to Fe, Al, and Mn oxides being the fraction presenting the highest values under both levels of arsenic pollution (25 and 50 mg As kg^{-1}). The bioavailability

of As extracted by DTPA is depicted in Fig. 5, showing that the Fe-BCNC + PGPR amendments effectively decreased As bioavailability in soil. Specifically, the extractable DTPA-As was reduced by 52% due to the combined treatment of Fe-BCNC + PGPR, compared to the control, when the pollutant dose was 25 mg As kg^{-1}, and by 53% when As was dosed at 50 mg kg^{-1}. On the other hand, As retention in soil was significantly enhanced with the addition of PGPR and Fe-BCNC in comparison to the control, under both levels of As stress, achieving 40% and 47% for the doses of 25 and 50 mg kg^{-1}, respectively. The combined application of Fe-BCNC and PGPR showed the highest increase in the Fe–Mn bound As fraction for both the 25 and 50 mg kg^{-1} As polluted soils, being around 158% and 75% higher than its respective controls. Similarly, these amendments increased the organic-matter-bound As fraction by 9% and 76%, respectively. With the application of both Fe-BCNC + PGPR, As in soil was distributed in the following order F5 > F1 > F3 > F4 > F2, where F1 represents exchangeable As, F2 represents As bound with carbonates, F3 corresponds to As bound
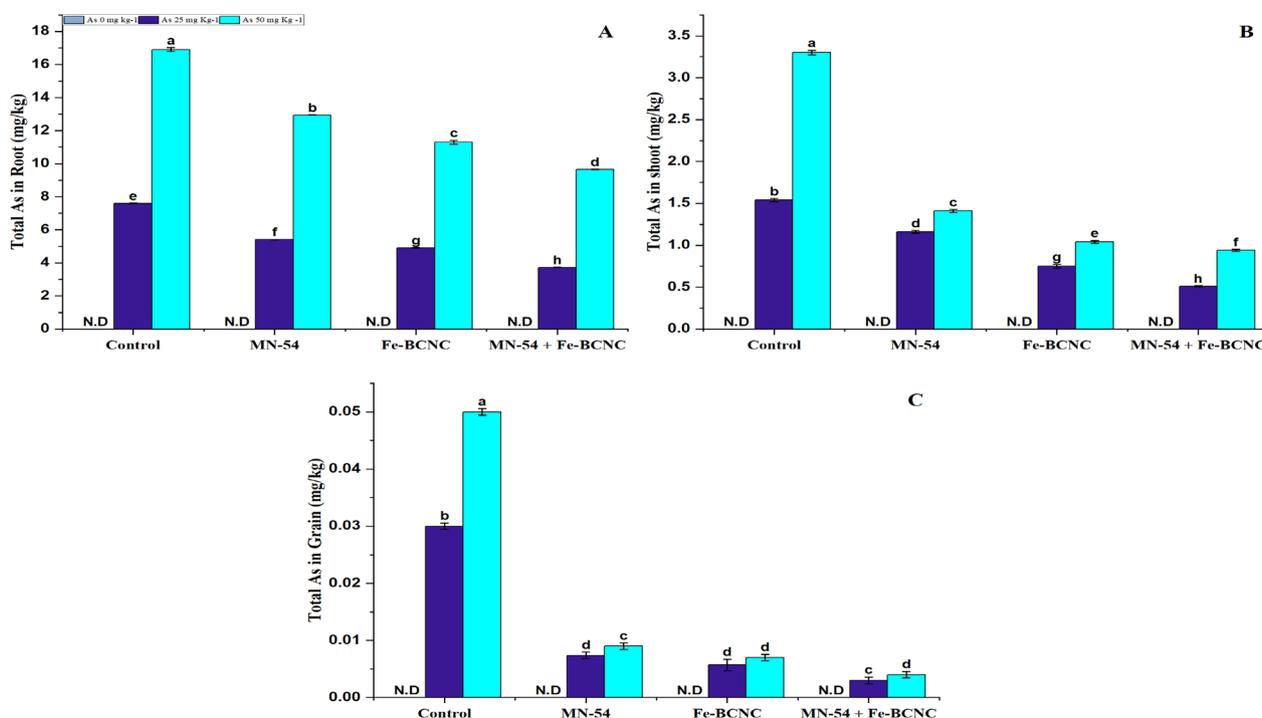
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Figure. 4. Effect of Fe-BCNC and PGPR on arsenic accumulation in *H. vulgare* under normal and stressed conditions, with different doses of As indicated using different colors for histograms. Columns are representing mean values ($n = 3$), whereas bars are indicating the standard error (S.E). Different letters are representing the significant difference among all mean values according to Duncan multiple range test under CRD at P ≤ 0.05. N.D.: not detected (below the detection limit). MN-54: plant growth promoting bacterial strain *Bacillus* sp.; Fe-BCNC: iron-biochar nanocomposite; MN-54 + Fe-BCNC: plant growth promoting bacterial strain *Bacillus sp.* and iron-biochar nanocomposites

with iron/manganese oxide, F4 depicts As bound with organic matter, and F5 indicates the residual fraction of As in soil.
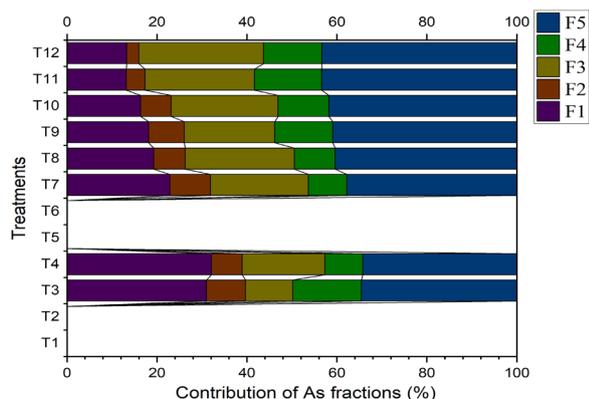
Translocation, health risk and cancer risk assessment

The TF score represents the capacity of the plant to transport metals from root to shoot. Figure 6 displays the TF_{As} values in *H. vulgare*. The data suggest that TF_{As} increased with the accumulation of metal in plant parts as the applied As dosage increased. Although the values of TF, BCF and BAF for As were below 1 in all cases, the accumulation of arsenic in grains may cause health threats to humans and animals. The health risk index (HRI) and integrated lifetime cancer risk (ILTCR) for As were calculated under the different treatments and stress conditions to determine the health risks associated with the ingestion of *H. vulgare*, with the results being depicted in Fig. 7. The HRI values of contaminated samples were above 1, but the combined application of Fe-BCNC and PGPR decreased the scores under both arsenic stress levels (25 and 50 mg As kg^{-1}), at levels around 92% and 94% lower than the respective controls. The minimum ILTCR values were recorded under the combined application of Fe-BCNC
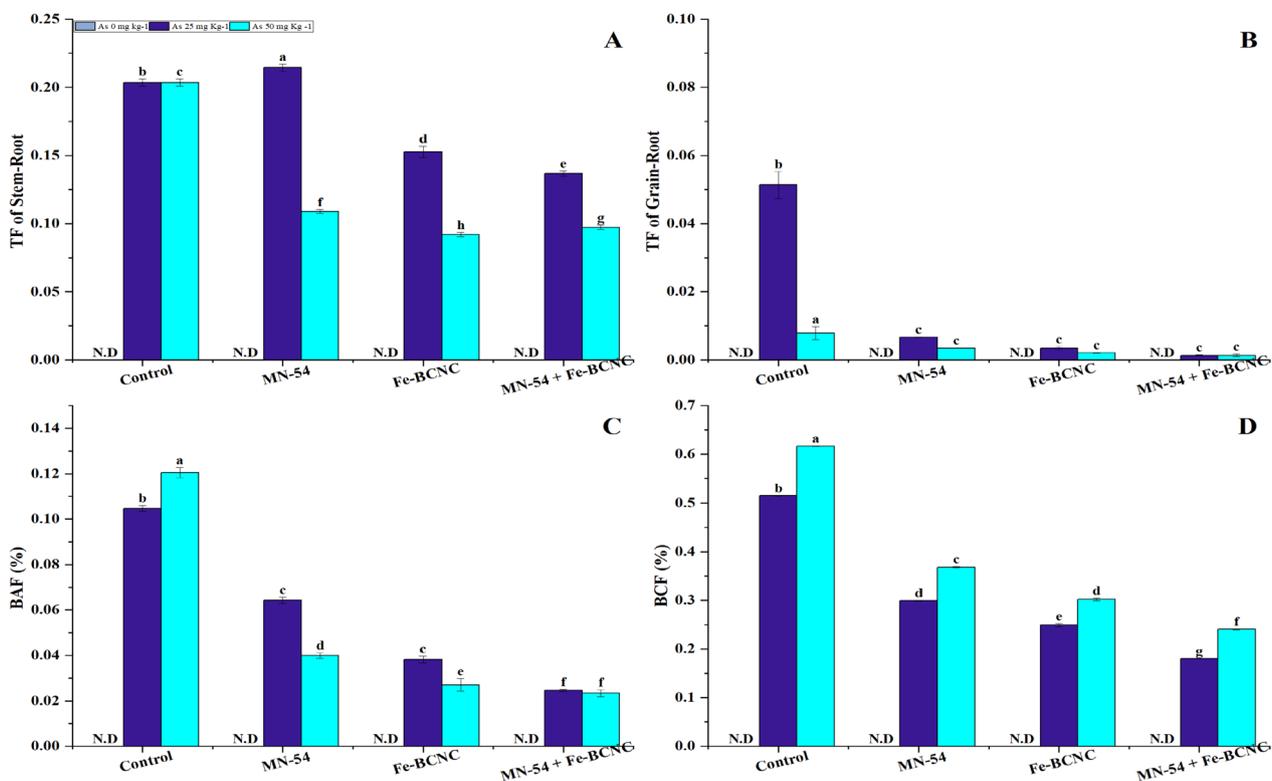

Fig. 5 Effect of Fe-BCNC and PGPR on geochemical arsenic fraction. F1 represents exchangeable As, F2 represents As bound with carbonates, F3 corresponds to As bound with iron/*manganese oxide*, F4 depicts As bound with organic matter, and F5 indicates the residual fraction of As in soil. T1: control, T2: Fe-BCNC; T3: As 25 mg/kg; T4: As 50 mg/kg; T5: PGPR; T6: PGPR + Fe-BCNC; T7: PGPR + As 25 mg/kg; T8: PGPR + As 50 mg/kg; T9: Fe-BCNC + As 25 mg/kg; T10: Fe-BCNC + As 50 mg/kg; T11: Fe-BCNC + PGPR + As 25 mg/kg; T12: Fe-BCNC + PGPR + As 50 mg/kg

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Figure. 6. Effect of Fe-BCNC and PGPR on the values of bio-concentration factor (BCF), bioaccumulation factor (BAF), and translocation factor of stem-root and geain-root (TF) of *H. vulgare*, with different doses of As indicated using different colors for histograms. The values are presented as mean ± standard error ($n = 3$). The values sharing the same letter(s) in bars have statistically non-significant differences with each other at P ≤ 0.05 under CRD. N.D.: not detected (below the detection limit). MN-54: plant growth promoting bacterial strain *Bacillus* sp.; Fe-BCNC: iron-biochar nanocomposite; MN-54 + Fe-BCNC: plant growth promoting bacterial strain Bacillus sp. and iron-biochar nanocomposites
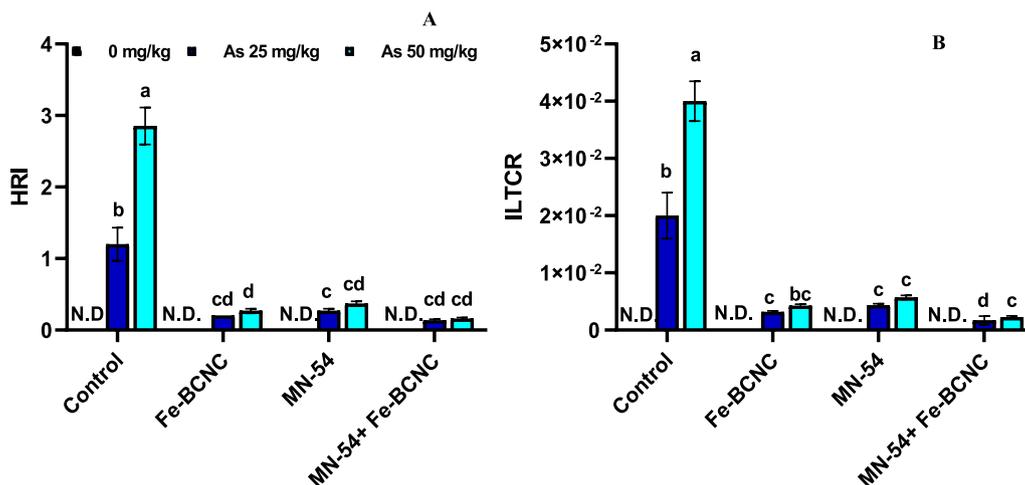

Figure. 7. Effect of Fe-BCNC and PGPR on the values of health risk index (HRI) and integrated lifetime cancer risk (ILTCR). The values are presented as mean ± standard error ($n = 3$). N.D.: not detected (below the detection limit). The values sharing the same letter(s) in bars have statistically non-significant differences with each other at P ≤ 0.05 under CRD. MN-54: plant growth promoting bacterial strain *Bacillus* sp.; Fe-BCNC: iron-biochar nanocomposite; MN-54 + Fe-BCNC: plant growth promoting bacterial strain Bacillus sp. and iron-biochar nanocomposites

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and PGPR, reaching levels of 2×10^{-4} and 2×10^{-3} for the 25 and 50 mg kg^{-1} doses of pollutant in the receiving soils, being 99 and 94 times less than in the unamended edaphic materials.

Principal component analysis

PCA was conducted to analyze the impact of all the treatments included in this study. The score (a) and loading plots (b) of PCA on some important attributes of *H. vulgare* plants are presented in Figure S1 (Supplementary Material). The dots on loading plot represents different treatments of study while the vectors on score plot represents the studied parameters. All the components, i.e., PC1 (Dim 1) and PC2 (Dim 2) showed maximum contribution and were considered for the 92.5% of total variances in the dataset, of which PC1 contributed 78.8% and PC2 contributed 11.7%. All the treatments were scattered and separated successfully. This distribution of treatments clearly indicated that Fe-BCNC and PGPR amendments, both individually and in combination, significantly improved the studied attributes of barley plants in both normal and As-contaminated soil compared to the control (Figure S1a). Arsenic contaminated treatments without Fe-BCNC and PGPR (3, 4) were more displaced from the treatment compared to the control (1). Similarly, the As-pollution treatments with the addition of Fe-BCNC and PGPR, alone or in combination (5–12), showed significant differences compared to the contaminated treatments (3, 4) (Figure S1a). The first group of variables with which PC1 is positively correlated include variables such as NRo, NSh, Chlb, Chla, SPAD, Chl c, PSh, NG, and PG. Contrary, a significant negative correlation was found for the PC1 variable with the variable aligned with PC2 (Figure S1b).

Discussion

Iron biochar nanocomposites characterization

According to Cui et al. [30], the SEM image of their prepared nanocomposites showed rough and porous structure with round shape iron particles at the surface. They also predicted that Fe could not only stick to the surface, but also penetrate the inside of BMN and react with biomass to produce iron oxides with high dispersion. Their FTIR analysis also confirms the deposition of Fe–O at the surface of BMN. The SEM image of Fe–O biochar nanocomposites shows the rough and porous surface of biochar which provides the broad contact surfaces for Fe_3O_4 particles to settle on [44]. The drop in the OH- group might be attributed to the formation of metal–oxygen bonds. The vibration peak at 1096, 802, and 461 cm^{-1} was attributed to Si–O–Si bonds [140, 60, 62]).

Agronomic, physiological and chemical attributes

The present study showed that arsenic pollution caused a negative outcome on growth and physiology of *H. vulgare*. In the studied conditions, arsenic showed hazardous effects on crop growth and physiology, with potential to affect crop yield. The As-contaminated soil samples showed reduction of agronomic parameters, with *H. vulgare* physiological attributes also compromised. In this regard, As is widely recognized to be poisonous to plants even at low-to-moderate concentrations [21, 100, 118]. Plant growth reduction in response to As exposure could be associated with restricted intake of essential nutrients [2], decreased gaseous exchange activity [115], formation of reactive oxygen species [21, 102, 113], consequently leading to ionic toxicity [110]. For rice plants it has been shown that the increased accumulation of As also affects the chlorophyll content, with the degree of loss reached significantly depending on the types of As, but any case finally reducing the rate of photosynthesis, resulting in decreased root and shoot development and grain output [93]. In addition, Rizwan et al. [99] and Burachevskaya et al. [23] concluded that plants grown in As and Cd contaminated soils had low levels of chlorophyll contents, which was largely linked to its synthesis and due to deficiency of Mg and Fe. Li et al. [61] and Berenjani et al. [20] indicated that oxyradicals are produced by heavy metal stress, causing membrane injury, and molecules linked to it such as chlorophyll pigments, and the alterations induced by heavy metals would also include decrease in size and number of chloroplasts and disorganization of thylakoids and grana structure. Also relevant, it is known that arsenic competes with phosphate for root absorption due to its molecular similarities [4, 117].

Plant growth promoting bacteria (PGPR) and Fe-BCNC collectively enhanced agronomic and physiological parameters of *H. vulgare* in unpolluted as well as in As tainted soils. Previously, the findings of various researchers indicated that the use of biochar nanocomposites and PGPR improves plant growth under abiotic stressed conditions [16, 43, 72]. Biochar nanocomposites containing Mg and Mn caused enhanced morphological and physiological attributes of safflower and maize crops under abiotic stresses [40]. Çığ et al. [28] analyzed the effectiveness of biochar and PGPR on the growth of wheat, as well as post-harvest soil properties, and concluded that PGPR improved the overall wheat biomass, root fresh and dry weight along with increased germination rate, nutrient elements and pH. The use of nano-silica modified biochar has been considered positive to boost plant development through several mechanisms, including enhanced pH and cationic exchange capacity (CEC) of the soil, better nutrient contents and metal ion adsorption [13]. Silicon-induced growth augmentation in response to As

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stress is linked to the modulation of nutrient absorption, antioxidant enzymes, metal speciation and soil pH alterations. Silicon reduces metal stress via tweaking metal speciation, soil pH and the creation of inorganic crystals on the surface of biochar. In the current experiment, the XRD results confirm the presence of SiO_2 and Al/Si functional groups on the surface of biochar. SiO_2 has shown the highest affinity toward As (III), hence reducing its plant uptake, which ultimately boosted the growth and physiology of plants [13].

Regarding previous studies supporting the positive effects of growth promoting bacteria, as shown in the current research, Dutta et al. [33] shown that the application of *Klebsiella pneumoniae* enhanced the growth and physiology of black gram plants under metal toxicity, whereas Rizvi et al. [98] reported that, after the exposure of maize to lead and copper pollution, the inoculation by *Azotobacter chroococcum* boosted the chlorophyll pigments and reduced heavy metals crop uptake.

According to Chen et al. [27], increased C-associated functional groups like carboxyl, aldehydes and increased adsorption potential of magnetic Fe made it an excellent adsorbent for toxic pollutants. Nano-particles are also reported for improved plant growth and decreased heavy metals uptake and as an efficient remediation tool under stress environmental conditions [49, 73, 90, 99, 119, 132]. In the current study, the decrease in As content and the improvement in crop growth might be due to the various direct and indirect mechanisms induced by magnetic-biochar-like As complexation with functional groups present on the biochar surface, which reduced the mobility and bioavailability of the pollutant (as could happen for other potentially toxic heavy metals/metalloids). Of relevance could be electrostatic attractions, with negatively charged surface of biochar attracting positively charged forms of different pollutants, reducing their bioavailability, as previously noted for other adsorbents [24]. Majumdar et al. [71] have conducted an experiment on iron-doped biochar composites to reduce arsenic stress and improve plant nutrients. They conversed about the significance of changing the oxidation state of Fe in improving P and N availability and assimilation in plants. They conversed that both crystalline and non-crystalline Fe (III) (hydro)oxides served as terminal electron acceptors for organic matter mineralization and N transformation. Reducing Fe^{3+} may convert NH^{+4} to NO^{-2}, leading to the creation of dissolved organic nitrogen. In the presence of a solid P mineral (e.g., rock phosphate), reductive dissolution of Fe (III) (hydro)oxides on the biochar surface might result in the production of a soluble P phase.

The current study showed that arsenic stress reduces the N and P content in different parts of *H. vulgare*. However, the application of Fe-BCNC and PGPR improved the nutrient contents in plants as well as in soil. In this regard, Li et al. [63] explored the significance of changing the oxidation state of Fe in increasing N, P, and S availability and plant uptake, finding that reducing Fe^{3+} can convert NH^{+4} to NO^{2-}, promoting the dissolution of organic nitrogen. Focusing on phosphorus, in the presence of solid P minerals, the reductive dissolution of Fe (III) (hydro)oxides occurring on the biochar surface may lead to the formation of a soluble P phase, while it has been observed that interactions between humic compounds and biochar surfaces could yield soluble Fe-humic complexes, and, consequently, this process could accelerate the release of phosphorus from its solid state into the soluble phase by inhibiting P co-precipitation with Fe(III) (hydr)oxides. Given that both arsenic (As) and phosphorus (P) are classified within the main group VA in the periodic table of elements, the phosphate anion (PO_4^{3-}) emerges as a potent ligand, competing with As for adsorption sites due to their analogous outer electronic configurations. The abundance of phosphorus creates a competition between As and P ions which play a crucial part in reducing As uptake in different plant parts [38]. According to the Aborisade et al. [1] addition of biochar plays a crucial role in elevating soil pH. pH is a critical factor in regulating toxic metal transport in soil. The increment in soil pH can be attributed to the changes in iron-biochar nanocomposites and the release of the hydroxyl group, as shown in Eqs. (1).

$$4Fe^{2+} + 4H_2O + O_2 \rightarrow 4Fe^{3+} + 2H_2O + 4OH^-$$
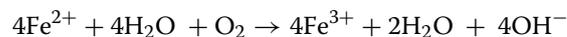
Increase in soil pH might promote the formation of metal hydroxides, carbonates, and phosphates, which could greatly enhance metal stabilization. They also stated that the reduction in the level of metal in soil, plant shoots and roots could be due to negatively charged particles on the surface of the adsorbents, which can increase the electrostatic attractions for metal ions and promote the formation of complexes or precipitates. The increase in soil organic matter due to the treatments may have increased the OH concentrations and negative charges, thereby enhancing the remediation efficiency. For the future, additional research would be needed with the specific objective of shedding further light on the exact mechanisms behind improved growth and nutrient absorption in plants using Fe modified biochar under As stress. Rhizobacteria, which promote plant development, increase phosphorus availability by mineralizing and solubilizing rock phosphate complexes (organic and inorganic phosphorus). Plant growth-promoting rhizobacteria increase plant development directly by phytostimulation and bio-fertilization, and indirectly through biopesticides or bio-control agents [124].

On the other hand, it has been suggested that PGPR could help in crop growth promotion by producing phytohormones [7], fixing dinitrogen into plant available forms [124], accelerating the availability and uptake of vital nutrients, solubilizing iron and phosphorous by making chelates, and producing organic acids, hence ensuring proper plant development and growth [103].
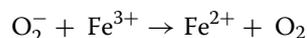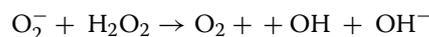
Grain quality attributes

The results of the current study have shown that although arsenic causes negative effects on grain quality of barley, the application of Fe-BCNC and PGPR is able to mitigate/correct it. Similarly, Ramzani et al. [94] found that the application of biochar and ferrous sulfate improved the composition of rice grains grown in soils with variate pH values. Higher nutrient availability and uptake due to the addition of plant growth promotor bacteria could explain the improved grain quality contents of *H. vulgare* grown in the presence of biochar and bacteria compared to commercial fertilizes, as reported earlier by Seleiman et al. [107]. Ramzani et al. [94] also indicated that biochar fertilizers are linked to improved mineral nutrition, resulting in a higher crude protein content. However, the detailed nutritional values of *H. vulgare* crops grown with iron-biochar nanocomposites and bacteria remain unexplored, with the current study being just a kind of starting point in this regard. In previous investigations, beneficial bacteria were used in wheat crops, resulting in a notable increase in both the crop's mineral content and total carbs [11], which might be due to the conversion of certain soil nutrients into bio-available forms through the process of mineralization. According to Pandey et al. [87] and Dhiman et al. [31], PGP *Bacillus* spp. significantly improved the nutritional qualities of *Amaranthus hypochondriacus* and increased the amount of specific chemical ingredients. Increased protein content is the outcome of a greater nitrogen content by the *Bacillus* isolates, which is used for protein synthesis in the plant. Indeed, the increase in chemical components might be attributed to bacilli strains' PGP activities. *Bacillus* isolates have been proven beneficial in increasing nitrogen, phosphorous, and potassium concentrations in soil and their consumption by plants.

Antioxidant enzymatic activities

In response to abiotic stresses, superoxide anions are generated, and SOD decreases radical-induced stress in plants. Further, CAT neutralizes H_2O_2 by transforming it into oxygen and water molecules [70, 116]. Arsenic stress increases the antioxidants activity in *H. vulgare*, but it can be mitigated by means of the individual as well as the combined application of Fe-BCNC and PGPR. These results are consistent with those provided by Majumdar

et al. [71], who used iron oxide doped biochar for growth of rice plants under arsenic stress. These authors indicated that a thorough molecular assessment of arsenic-mediated stress on rice plants revealed the disruption of internal integrity caused by decreasing the activity of the cellulose synthase genes *CESA3* and *CESA4*. The down-regulation of these genes during arsenic stress leads to a diminished cellulose synthesis rate in plants, thereby destabilizing the inner configuration. Treatments with modified biochar mitigated arsenic toxicity in rice seedlings, enhancing cellulose synthesis. The transportation of arsenic to the upper plant tissues gradually diminished due to the formation of complexes with modified biochar, resulting in a reduced expression of antioxidant enzymes in the plant parts. Khan et al. [56] suggested that application of Fe-BC reduces antioxidants by reducing the impacts of heavy metals/metalloids by decreasing its translocation and binding it with different functional groups (such as some related with iron and silicon) of prepared biochar added to soil, consequently enabling wheat plants to improve nutrients uptake and photosynthetic pigments. In the current research, FTIR results prove the presence of those functional groups on biochar's surface taking part in As immobilization onto soil and reducing its translocation in plant parts, which ultimately reduces the ROS-induced oxidative stress. Aborisade et al. [1] reported that increasing the activities of antioxidant enzymes could aid in diminishing plants' oxidative stress and bolstering plant resilience by constraining the uptake of both Cd and Pb through the administered treatments. They also proposed the possible mechanism of action of their iron-biochar nanocomposites as follows:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$$

$$O_2^- + H_2O_2 \rightarrow O_2 + + OH + OH^-$$

$$O_2^- + Fe^{3+} \rightarrow Fe^{2+} + O_2$$

### Arsenic fractions and bioaccumulation in plant parts

In this study, both amendments increased the amount of arsenic retained in soil, which is in accordance with previous findings [18, 27, 66, 126], while the accumulation of As in plant parts was reduced after the application of these Fe-BCNC and PGPR amendments. In this regard, Lin et al. [64] tested the ability of ferro-manganese oxide biochar nanocomposites to bind arsenic to red soils, finding that soil As concentrations decreases up to 60% with the addition of their prepared nanocomposites. These authors suggested that the surface area of the adsorbents as well as functional groups present at the

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surface of nanocomposites are responsible for arsenic reduction in soil. Additionally, As ions on the surface of the above-mentioned mixed oxides can coordinate with various surface functional groups (–OH, –COOH). In soil, As (III) and As (V) may form surface complexes with a variety of oxides, including Fe, Mn, and Al, reducing As mobility and bioavailability. Bacteria also affect metal bioavailability in soils, promoting chelation, acidification, and precipitation. As an example, organic acids generated by microbes and plant roots abate soil pH and help in metal ion sequestration [76].
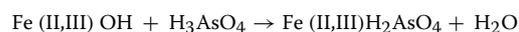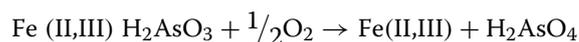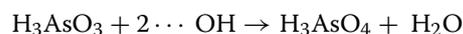
Heavy metal removal mechanisms include complexation, ion exchange, precipitation, π-interaction, and electrostatic interaction. In contrast, the removal of organic contaminants involves electrostatic attraction, hydrogen bonding, π-interaction, complexation, and pore-filling interaction [88]. Biochar possesses a variety of oxygen-containing functional groups that have the potential to form complexes with heavy metals and participate in cation exchange processes with metal cations situated on its surface. These functional groups include carbonyl, carboxyl and phenol [84, 108]. As biochar oxidizes over time, fresh reactive sites are created on its surface, facilitating the immobilization of heavy metals and eventually reducing their uptake by plants [81]. In the present study, *H. vulgare* plants may have had lower metal absorption rates because of the applied amendments, which would have increased phyto-stabilization effectiveness and decreased health hazards. This may correspond to an increase in soil organic matter (OM) and iron contents in response to the application of iron-impregnated biochar. The results showed that the addition of biochar caused a shift in As fractions from exchangeable, carbonate, and OM-bound As to Fe–Mn-oxides As fractions. Modified biochar exhibits the capability to impede arsenic absorption via both direct and indirect pathways. Direct interactions encompass processes such as electrostatic attraction, ion exchange, precipitation and complexation [46], Kumar and Bhattacharya, [58]), while factors such as soil organic carbon, mineral dissolution and soil pH may indirectly contribute to limiting arsenic uptake [74].

Previous investigations revealed that certain nanoparticles and nanocomposites may enhance soil pH, thus restrain heavy metals in soil, as pH has a marked influence in the adsorption of toxic metals [1, 30, 32, 35, 39, 47]. Cui et al. 2019 [30] reported that BMN nanocomposites have shown the ability to oxides some of As (III) to As (V) in sandy soil. The study revealed that the application of BMN to soil could sparingly increase the pH of soil which could be rationalized due to the formation of complexes between Fe and As by reducing H^+ ions in soil. Moreover, BMN has significantly lowered the available As in soil, which indicated the effective reduction in

bioavailability of As. They have also proposed the three possible pathways of arsenic immobilization. Firstly, As (III) could be adsorbed on the surface of iron oxides generated inner-sphere bidentate-mononuclear species:

$$Fe\,(II,III)\,OH\,+\,H_3AsO_3 \rightarrow Fe\,(II,III)\,H_2AsO_3\,+\,H_2O$$
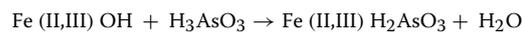
Secondly, some part of As (III) was oxidized to As (V) by reactive oxygen:

$$H_3AsO_3 + 2 \cdots OH \rightarrow H_3AsO_4\,+\,H_2O$$

$$Fe\,(II,III)\,H_2AsO_3\,+\,{}^1\!/_2O_2 \rightarrow Fe(II,III)\,+\,H_2AsO_4$$

$$Fe\,(II,III)\,OH\,+\,H_3AsO_4 \rightarrow Fe\,(II,III)H_2AsO_4\,+\,H_2O$$

Lastly, the produced As (V) was readily adsorbed onto iron oxide nanoparticles of BMN.

Similarly, Saif and Khan [105] reported that rhizobacterial inoculation in chickpea grown in Cr–Ni tainted soil reduced the metal uptake, while Kavamura and Esposito [55] and Ojuederie and Babalola [86] indicated that metals toxicity was reduced due to biosorption and accumulation ability of rhizobacteria. Other previous findings showed that siderophore-producing bacteria decontaminated HMs-polluted soils by binding to a wide range of toxic metals [52]. Microorganisms might involve extracellular and intracellular bioremediation techniques mainly biosorption and bioaccumulation. The cell walls of microorganisms may contain lipids, proteins and polysaccharides (cellulose and alginate) which contain a variety of functional groups such as hydroxyl, sulfonate, amino, carboxyl and phosphate. These groups provide great potential as binding sites [8]. Several studies stated positive effects of the PGPR, organic amendment and iron oxide nanoparticles for immobilization of heavy metals to bolster the growth and yield of maize under heavy metals stress [5, 29, 48, 79].

Microorganisms employ various strategies to combat arsenic toxicity within their environment. These mechanisms encompass: (1) the exclusion of As (III) from cells; (2) the sequestration of intracellular As (III) through many metal-binding peptides such as phytochelatins (PC), glutathione (GSH), and metallothioneins (MT); and (3) the conversion of arsenic into less harmful forms. In the presence of As (III), microorganisms derive cellular energy through the oxidation of As (III), utilizing it as an electron donor [129]. Arsenite-oxidizing bacteria contain arsenite oxidase, and with the help of this enzyme As (III) is converted into As (V), which is then adsorbed onto the different amendments having iron, and ultimately reduces the mobility, bioavailability and exchangeable fraction of As [54]. With no previous study focused on the role of integrated use

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of PGPR and iron-modified biochar in the immobilization of As and growth promotion of crops, in the current research the results indicated that the combined use of arsenic tolerant PGPR and Fe-BCNC boosted the growth and yield parameters of *H. vulgare* and immobilized the heavy metals. The possible mechanisms behind the reduced assemblage and translocation of heavy metals by plants through Fe-BCNC might be the surface adsorption of As (V) on the Fe, P and Si present in functional groups of the nano-bio-composites, this taking place after the bio-conversion of As (III) into As (V). Other mechanisms would involve surface adsorption, chelation, complexation or precipitation [122]. The increase in growth and yield attributes as well as improvement in physiology of *H. vulgare* might be helped by the surface adsorption capacity of the modified biochar (through complexation), which is a key mechanism typically induced by biochar materials [67].

Bioaccumulation and health risk factors

The present study depicted that the translocation of arsenic in plant parts could pose heath risk in untreated and contaminated soils, while the application of different amendments has significantly reduced the translocation factor as well as the possible health and cancer risks. These results are aligned with those by Yang et al. [131], who reported the reduction of the bio-concentration factor along with the health and carcinogenic risks associated to the consumption of rice grown in arsenic and cadmium polluted soils. They suggested that the addition of biochar with nanoscale zero-valent iron reduced the heavy metal translocation to aerial parts of rice plants, which could be due to the certain properties of the amendment such as higher number of active adsorption sites present at the surface. As previously shown, arsenic can be mainly adsorbed to Fe oxides and biochar due to electrostatic and specific adsorptions [125]. In the current study, the peaks in the FTIR spectra showed the iron–oxygen linkage (Fe–O) on biochar surface. This adsorption leads to lower accumulation of the pollutant into plant parts, which ultimately reduces the health risk associated with the consumption of contaminated food crops. To note that heavy metal-tolerant microorganisms have developed different mechanisms to detoxify metals from environmental compartments such as soil and water. Those mechanisms include (1) binding with extracellular materials such as cell wall, with a vast number of cationic metals binding to anionic functional groups on cell surfaces; (2) siderophore production; (3) biosurfactants; (4) sequestration by metallothioneins or similar proteins; (5) methylation (Pepper et al., [89]).

Conclusions

In this study a novel Fe-BCNC composite together with PGPR *Bacillus* sp. were used to lower As accumulation in *H. vulgare* plants. The integrated application of Fe-BCNC and *Bacillus* sp. positively influenced agronomic, physiological and biochemical aspects of *H. vulgare*, mitigating deleterious effects due to As. The modification of Fe-BCNC achieved with iron chloride was confirmed by FTIR and XRD analyses revealing the presence of higher Fe content at the surface of the biochar. The positive effects could be due to the synergistic action of PGPR (lowering As toxicity) and Fe-BCNC (which could bind As on surface functional groups and oxidize As (III) into As (V)). Having also verified that Fe-BCNC had the potential to convert unstable [As (III)] into stable As [As (V)], Fe-BCNC and PGPR application could be used as an effective approach for mitigating As-induced stress in *H. vulgare* through phyto-stabilization, ultimately leading to a decline in health risks associated to the food chain. Moreover, taking into account the potential of biochar to alleviate As-induced crop losses in outdoor agriculture, the use of plant growth promoting bacterium (*Bacillus* sp.) in conjunction with nano-organic inputs could provide a long-term, and cost-effective solution to nutritional scarcity along with remediation measures in developing countries. Overall, the results from this research could help in developing bio-remedial strategies for crop cultivation in arsenic polluted agricultural areas, which can be seen as a matter of environmental and social relevance. However, additional investigation is integral to scrutinize the potential of multi-traits microbes and nanocomposites for As (III) remediation and plant growth promotion under different field and environmental conditions with other fertilizer strategies and its feasibility for large-scale application. The mechanistic insights of this research may help in developing bio-remedial strategies for crop cultivation in As(III) polluted agricultural areas.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-025-01239-x.

Supplementary Material 1.

Data availability

Data are provided within the manuscript or supplementary information files.

Declarations

Competing interests

The authors declare no competing interests.

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