



Field-scale evaluation of nanoscale zero-valent iron and biochar coupled with phytoremediation for *in situ* stabilization of polluted soil

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ABSTRACT

Biochar and zero-valent iron nanoparticles (nZVI) are promising amendments for stabilizing polluted soils due to their cost-effectiveness and environmental benefits. However, their field-scale application and impact on the soil-plant system remain underexplored. This work evaluated a pilot-scale experiment using As- and metal-polluted soil, where 7 % wt biochar and 1 % wt nZVI were applied individually and combined over 60 days. Soil properties, pollutant mobility, and the physiological response of *Brassica juncea* were assessed. The amendments significantly reduced metal mobility in the soil (up to 73 %, 63 %, 59 % and 81 % for Cu, Zn, Cd and Pb, respectively), which correlated with a decrease accumulation in roots. However, biochar-treated soil resulted in an elevated As concentration in the aerial part of the plants (37 % increase), while the nZVI application led to a notable reduction (41 % decrease). Plants grown in biochar-treated soil exhibited reduced levels of H₂O₂ and MDA (34 % and 21 % decrease), whereas leaves of plants growing in nZVI-treated soil revealed a higher H₂O₂ concentration (27 % increase). Interestingly, the combined application of biochar and nZVI mitigated the oxidative stress induced by the Fe-based amendment. Furthermore, the highest biomass production was observed in plants grown in biochar-treated soils, both alone and combined with nZVI, which coincided with the highest proline accumulation in leaves. In conclusion, these findings suggest that biochar and nZVI co-application emerges as a reliable strategy to achieve an adequate balance between the simultaneous immobilization of As and metals and promoting satisfactory plant growth without relevant symptoms of stress.

1. Introduction

Residues from industrial activities such as mining, coal burning, smelting, or agricultural fertilizers have contributed to increasing metal (loid) concentrations above natural soil backgrounds. Metal(loid) contamination is a problem worldwide. In this context, it is estimated that there are more than 5 million contaminated sites affected by this type of pollutant [1,2]. In addition, deindustrialization has left a legacy of many contaminated brownfields [3], which are often located in urban downtown areas absorbed by expanding towns and cities. These sites are commonly the only available option for urban and greenfield development [4].

For soil remediation, chemical stabilization amendments such as biochar have been combined with novel approaches like environmental

nanotechnology [5-7]. Biochar is a solid carbonaceous material derived from the thermal conversion of biomass feedstocks, and it has attracted attention due to its porous structure, variety of functional groups, high specific surface area, cation exchange capacity, and alkaline pH [8-10]. On the other hand, Fe-based nanoparticles [11] have gained importance as a soil amendment in recent years, especially zero-valent iron nanoparticles (nZVI) [12]. In this regard, nZVI are relatively inexpensive and non-toxic and they show greater effectiveness than microparticles due to their higher reactivity and specific surface area [13]. The effectiveness of nZVI for soil remediation is variable and depends on the type of pollutants present as various mechanisms, including sorption, complexation, (co)precipitation, and surface-mediated chemical reduction, may take place [14]. However, given that nZVI do not supply nutrients to degraded soil, they are usually combined with organic-type

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amendments [15,16].

Recent advancements have underscored the synergistic potential of combining biochar with nanoscale zero-valent iron (nZVI), particularly through the creation of iron-modified organic amendments [17–19]. The selection of nZVI is primarily driven by its exceptional surface area and high reactivity, attributes that notably enhance its effectiveness compared to traditional iron-based amendments. Various methodologies have emerged, including direct soil mixing of nZVI and biochar [5, 7,20], biochar modification through ferric ion reduction or impregnation [21,22], and composite synthesis via one-step pyrolysis of biomass and iron ore [23]. These strategies address the limitations associated with biochar application alone, as biochar frequently exhibits restricted efficacy in immobilizing metalloids such as arsenic, and may, under certain conditions, even increase their mobility by elevating soil pH and levels of dissolved organic matter, which competes with this metalloid for sorption sites [24]. Combining biochar with nZVI can mitigate these issues by leveraging the porous structure of biochar to stabilize nZVI, thereby extending its reactivity duration and enhancing its contaminant immobilization capacity [23]. Despite the growing body of research on biochar-nanoparticle composites [25], significant knowledge gaps remain concerning their underlying synergistic mechanisms and their long-term impacts on soil health and vegetation performance, particularly in field scenarios involving complex contamination by multiple pollutants [26–28]. Therefore, additional research is crucial to elucidate these interactions thoroughly and optimize the practical deployment of nZVI-biochar amendments.

To bridge this knowledge gap, the novelty of this study lies in the integration of these amendments within a complex and realistic experimental framework. Conducted on a paradigmatic site severely contaminated with four metals (Cd, Cu, Pb, Zn) and one metalloid (As) [29], the soil was treated with biochar and nZVI, applied individually or in combination, alongside the phytoremediation plant *Brassica juncea* L. This species, capable of both phytoextraction and phytostabilization [30,31], enabled the simultaneous evaluation of multiple phytoremediation strategies in combination with chemical stabilization, thus broadening the typical scope of phytomanagement. The monitoring strategy was designed to be integrative, encompassing soil physico-chemical properties, contaminant dynamics, and detailed plant physiological responses—an aspect rarely addressed in similar studies. This comprehensive assessment provides a deeper understanding of amendment–plant–contaminant interactions under real field conditions, offering valuable insights for the design and optimization of scalable remediation technologies.

2. Materials and methods

2.1. Soil and amendment characterization

Soil contamination is a significant environmental challenge across Europe, particularly due to the widespread presence of former industrial sites that are currently abandoned or underutilized, commonly known as brownfields [32]. One example is the Nitrastur site, a brownfield located in northern Spain, where this experiment was conducted. Soil contamination at this site primarily originates from the historical disposal of pyrite ash and other industrial by-products generated by prolonged chemical and metallurgical activities [3]. These residues, deposited over several decades without proper environmental management, have resulted in significant accumulation metal(loid)s in soils, posing considerable risks to both environmental quality and human health [33]. For this study, several pits were excavated in areas where concentrations of As, Cd, Cu, Pb, and Zn exceeded the Regional Soil Screening Levels. Soil from these pits was thoroughly mixed using mechanical methods, and a composite sample was subsequently created from five subsamples for characterization (Table 1).

Biochar and nZVI were used as soil amendments. The former, obtained from oak wood pruning at 400 °C for 4 h (see Table 1 for main

Table 1

Characteristics of the polluted soil and biochar.

Parameters	Units	Polluted soil	Biochar
pH		7.8 ± 0.1b	8.1 ± 0.1a
TOC		100 ± 2b	743 ± 4a
TN	g.kg ⁻¹	2.1 ± 0.9a	0.9 ± 0.3b
As		80 ± 6a	2 ± 2b
Cd		16 ± 1	<DL
Cu		3546 ± 120a	41 ± 3b
Fe	mg.kg ⁻¹	183,333 ± 4,932a	533 ± 58b
P		300 ± 10a	280 ± 10a
Pb		2186 ± 6a	7 ± 1b
Zn		5373 ± 80a	18 ± 1b
As		<DL	<DL
Cd		1.7 ± 0.2	<DL
Cu	mg.kg ⁻¹	100 ± 10a	0.3 ± 0.1b
Fe		0.4 ± 0.1a	0.4 ± 0.1a
Pb		17 ± 2	<DL
Zn		431 ± 22a	2.1 ± 0.1b
Ca ²⁺		32 ± 3a	13 ± 1b
K ⁺		1.2 ± 0.1b	5.6 ± 0.9a
Na ⁺	cmol(+)kg ⁻¹	0.8 ± 0.1a	0.9 ± 0.2a
Mg ²⁺		1.2 ± 0.1b	1.6 ± 0.1a
Al ³⁺		2.3 ± 0.4a	1.6 ± 0.2a
CEC		38 ± 3a	23 ± 2b

For each row, different letters in diverse samples mean significant differences (n = 3, ANOVA; P < 0.05). <DL: below detection limit; TN: total nitrogen; TOC: total organic carbon; P: available phosphorus (Mehlich 3); CEC: cation exchange capacity. See Section 2.3 for methods.

properties), was provided by Biesca Ingeniería (Asturias, Spain). The biochar produced has a particle size of less than 1 cm, as it was sieved after production. The latter, namely NANOFE 25S, was supplied by NANOIRON s.r.o. (Czech Republic). The nZVI particles contain 14–18 % of Fe and 2–6 % of magnetite. They have an average size of around 50 nm, the suspension is strongly alkaline (pH = 11), and the active surface area is 20 m²/g (additional details are available at www.nanoiron.cz). The microstructural characterization of nZVI was measured in a JEOL JEM-2100F transmission electron microscope (TEM). For TEM observations, sample preparation involved the dispersion of the NPs in a suspension of water by means of sonication, and deposition on a holey carbon film-coated copper grid and subsequent drying.

Brassica juncea was selected as the plant species for this study due to its ability to tolerate and accumulate metal(loid)s, typically found at high concentrations in contaminated industrial soils [34]. Additionally, this species exhibits rapid growth and high biomass production, demonstrating remarkable adaptability to adverse and polluted conditions, thus making it an excellent candidate for phytoremediation applications [35]. *Brassica juncea* L. seeds were supplied by ROCALBA S.A (Girona, Spain).

2.2. Field experiment

To conduct the field experiment, an area with contaminated soil was selected, and its characteristics are presented in Table 1. The soil was then excavated and mixed properly, and the plots were delineated using concrete bricks to separate the treatments. In this context, four 1 × 1 × 0.5 m plots were constructed and approximately 1 t of polluted soil was deposited in each one. One plot was left untreated (S, as a control), and the other three plots were subjected to different amendment strategies, including nZVI (SN), biochar (SB) and the combination of both amendments (SNB). The proportions of each amendment used are shown in Table S1. In brief, the nZVI dose was 1 % and biochar 7 % w/w, including when the amendments were applied simultaneously. The application doses were selected based on previous works due to not only technical but also economical aspects [5,15].

After applying the treatments, the amended plots were left to stabilize for 15 days and then, *Brassica juncea* L seeds were sown at a rate of 1 g per plot, a dosage determined based on the supplier's

recommendations. During the stabilization period, the plots were watered once every two days, reaching 60 % of water holding capacity. Thereafter, they were not irrigated artificially again and were subjected to the same environmental conditions (minimum temperature 13.6 °C, maximum temperature 24.3 °C, and maximum precipitation 54.8 l/m² (AEMET, 2021) during the experiment (60 days).

In alignment with the growth of *Brassica juncea*, the monitoring plan was adjusted to take place just prior to the flowering stage in order to mitigate any physiological changes that could influence metal accumulation in the plants. Consequently, the monitoring period for this experiment was established at 60 days. To obtain intermediate data regarding plant growth, plant physiology, and pollutant accumulation, supplementary sampling of both the vegetation and the soil was conducted after 30 days.

For monitoring purposes, soil was sampled three times during the experiment. The first set of samples was taken just before sowing the seeds (T1) to evaluate the effects of the amendments prior to plant growth. The second set was collected after 30 days (T2) to monitor the combined effects of soil amendments and short-term plant growth. Finally, the third set was taken at the end of the experiment, after 60 days (T3), when the plants were harvested. A sampling protocol was drawn up for each collection time. First, a sampling squared mesh was designed within each plot, and random soil sampling was then performed by taking five individual increments, which were subsequently mixed to obtain a composite sample.

Furthermore, plant development was also monitored. At 60 days, photosynthesis was measured using a portable photosynthesis system (LI- 6400XT, LI-COR INC). Net photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were measured on 10 leaves per treatment. Measurements were performed *in situ* under midday weather conditions, and the atmospheric CO₂ concentration was set at 400 μmol . The plants were harvested on day 60. In this regard, all the biomass from each plot was collected and preserved on ice until reaching the laboratory. Roots and aerial parts were separated after sampling. Fresh (FW) and dry weight (DW) and length were determined after cleaning the roots using tap water and several distilled water baths. Finally, samples were frozen at –80 °C before analysis, except for a small amount, which was used for dry biomass measurements and pollutant/nutrient concentration analysis.

2.3. Soil analysis

Soil samples from each plot were collected using a composite sampling approach consisting of three individual increments. Specifically, three random subsamples were taken from each plot using an auger to a depth of 15 cm. These subsamples were then thoroughly homogenized to form a single composite sample per plot, with a total weight of approximately 1 kg. Then, in the laboratory, soil samples were air-dried, passed through a 2 mm sieve, and homogenized before analysis. To obtain the total concentrations of metal(loid)s, samples were subjected to digestion with aqua regia in a microwave oven (Milestone ETHOS 1, Italy). Subsequently, the extracts were analyzed using atomic emission spectroscopy with inductively coupled plasma (ICP-OES; Optima 4300 DV, Perkin-Elmer). A certified reference material, NIST SRM 2711a, Soil Montana II, was used for conducting the analytical quality control. The available concentrations of metals were determined by means of a toxicity characteristic leaching procedure (TCLP) following the USEPA Method 1311 (1992), and the concentrations were also determined by ICP-OES. Soil pH was measured using a METTLER TOLEDO multi-meter in a 1:2.5 water-to-soil extract. Total soil carbon (TC) and total soil nitrogen (TN) were measured in a total organic carbon analyzer (TOC-V CSH) (Shimadzu) equipped with a module for solid samples. Available P was determined by the Mehlich 3 method. Exchangeable cations (Ca^{2+} , K^+ , Mg^{2+} , Na^+ , and Al^{3+}) were extracted with 0.1 M BaCl_2 and concentrations were determined by ICP-OES. The effective cation exchange

capacity (CEC) was then calculated using the sum of exchangeable cation concentrations.

2.4. Plant analysis

Frozen plant samples were ground with liquid nitrogen using a cryogenic mill (6775 Freezer/Mill® SPEX SamplePrep) for subsequent analysis of parameters related to oxidative stress and photosynthetic pigments. On the other hand, dry biomass obtained by heating in the oven at 40 °C for 6 days was used for metal(loid) and nutrient concentration analysis.

2.4.1. Metal(loid) and nutrient concentrations in plants

To determine the concentrations of metal(oid)s (As, Cd, Cu, Zn Pb) and nutrients (Ca, K, Mg, P) in the roots and aerial part of the plants, 200 mg of dried samples was digested using 2 mL of H₂O₂ and 4 mL of HNO₃ in a microwave oven for 6 min, as described by Baragaño et al. [15]. The translocation factor (TF) was determined using the following equation:

$$TF = \frac{[\text{metal(loid)s}]_{\text{aerial part}}}{[\text{metal(loid)s}]_{\text{root}}} \quad (1)$$

2.4.2. Parameters related to oxidative stress

Chlorophyll and carotenoids were extracted from 100 mg of frozen leaves using 2 mL of 80 % acetone. The mixture was then centrifuged at 6000 g for 5 min before measuring absorbance at 663, 646, and 470 nm, and the pigment content was calculated using the equations described by Porra [36]. Pigment content was expressed in $\mu\text{g g}^{-1}$ fresh weight (FW).

$$\text{Chlorophylla (Ca)} = 12.21\text{Abs}_{663} - 2.81\text{Abs}_{646} \quad (2)$$

$$\text{Chlorophyllb (Cb)} = 20.13\text{Abs}_{646} - 50.3\text{Abs}_{663} \quad (3)$$

$$\text{Carotenoids} = \frac{1000\text{Abs}_{470} - 3,27\text{Ca} - 104\text{Cb}}{229} \quad (4)$$

Chlorophyll fluorescence was measured with an OS1-FL portable fluorometer (Opti-Sciences) on the second fully expanded leaf, previously dark-adapted for 15 min. The Fv/Fm ratio was determined following the method described by Demmig-Adams and Adams [37].

Hydrogen peroxide (H₂O₂) content was determined following the method described by Fernández et al. [38]. First, 100 mg of leaves or roots was homogenized with 2.5 mL of 0.1 % trichloroacetic acid (TCA), and centrifuged at 12000 g for 15 min. Next, 0.5 mL of supernatant was mixed with 0.5 mL of 100 mM phosphate buffer (pH 7.6) and 1 mL of 1 M KI. Then, the absorbance was measured at 390 nm, and the H₂O₂ content was calculated from a standard curve and expressed as $\mu\text{mol g}^{-1}$ FW.

Lipid peroxidation was determined in leaves and roots following Fernández-Fuego et al. [39], by measuring the concentration of malondialdehyde (MDA). First, 100 mg of frozen leaves, stems and roots was mixed with 2 mL of 0.1 % TCA. The mixture was then centrifuged at 12000 g for 15 min, and a 0.5 mL aliquot of the supernatant was added to 2 mL of 20 % TCA with 0.5 % thiobarbituric acid. The samples were heated at 95 °C for 25 min and cooled in an ice bath. Finally, the absorbance of the supernatant was measured at 532 nm ($\epsilon = 155 \text{ mM}^{-1} \text{ cm}^{-1}$) and 600 nm for correcting non-specific turbidity. MDA content was calculated using the Lambert-Beer equation and was expressed as nmol g^{-1} FW.

$$MDA = \frac{\text{Abs}_{532} - \text{Abs}_{600}}{\epsilon} \quad (5)$$

The method described by Khedr et al. [40] was used to determine the proline content of leaves and roots. To this end, 100 mg of leaves or roots was homogenized with 1.5 mL of 3 % (w/v) sulfosalicylic acid and

centrifuged at 10000 g for 10 min. Next, 0.5 mL of supernatant was mixed with 2 mL of acid ninhydrin and 2 mL glacial acetic acid (1:1, v/v) and heated in a water bath at 100°C for 1 h. Samples were cooled in ice and 2.5 mL of toluene was added to the mixture. After the generation of two phases, the absorbance of the upper phase was measured at 520 nm. The proline content was calculated using a calibrating curve obtained and expressed as $\mu\text{mol g}^{-1}$ FW.

The commercial sources and purity grades of the reagents used for these determinations are shown in Table S2.

2.5. Statistical analysis

All analytical determinations were performed in triplicate. The data obtained were statistically treated using the SPSS program version 27.0 for Windows. Analysis of variance (ANOVA) and test of homogeneity of variance were performed. In the case of homogeneity, a post hoc least significant difference (LSD) test was performed. When there was no homogeneity, Dunnett's T3 test was carried out. A correlated bivariate analysis was also carried out by means of Pearson correlation.

3. Results and discussion

3.1. Evolution of total concentrations of As, Cu, Cd, Fe, P, Pb, and Zn

The total concentrations of the elements of interest did not vary significantly from the beginning to the end of the experiment, with few exceptions, indicating that no substantial changes occurred due to their potential extraction from the soil by *Brassica juncea* (Table S3). The total Fe concentration increased at the start of the experiments involving nZVI, which was expected due to the addition of Fe. However, Fe concentrations remained stable over time, suggesting that Fe losses through leaching or plant uptake were negligible. In contrast, the total metal (loid)s contents were compared across treatments to ensure that changes in the available concentrations of these elements (see Section 3.4) were

not a result of dilution effects caused by the addition of the amendments. This was generally the case, except for Cu, which exhibited significant differences between treatments at both the beginning and the end of the experiment. These differences can be attributed to soil heterogeneity at the site, as previous studies have reported high variability in Cu concentrations in soils due to the presence of diverse residues with varying Cu contents [3,33]. Finally, variations in P concentrations were observed at the final sampling, likely due to plant uptake of this element. This could explain the decrease in P levels in certain treatment, as plant growth was not uniform across all treatments.

3.2. Effect of amendments on pH, TOC, TN, and available P

The pH values remained relatively stable throughout the experiment, except in the SN treatment, where significant variations were observed. Notably, at both 0 and 60 days, soil amended with nZVI exhibited the highest pH values among all treatments (Fig. 1A). This initial increase can be attributed to the strongly alkaline nature of the nZVI suspension (pH = 11), which contrasts with the baseline soil pH (pH = 7.7), as previously reported by Gil-Díaz et al. [41]. Moreover, the addition of nZVI alters the acid-base balance in the soil by promoting the oxidation of Fe^0 to $\text{Fe}^{2+}/\text{Fe}^{3+}$, a process that consumes protons or generates hydroxide ions, thereby increasing pH. These redox transformations also contribute to the development of more reducing conditions in the soil, potentially affecting the speciation and mobility of nutrients and contaminants [42].

Interestingly, at day 30, the soil pH of the SN treatment declined to levels similar to the initial value, but increased again by day 60 (Fig. 1A). This non-linear trend may be related to the progressive oxidation and aggregation of nZVI in the soil matrix, processes that can alter surface reactivity and proton exchange capacity over time [43]. This interpretation is supported by the selected area electron diffraction (SAED) pattern of nZVI (Figure S1), which revealed the presence of concentric rings corresponding to crystalline iron oxide phases, such as

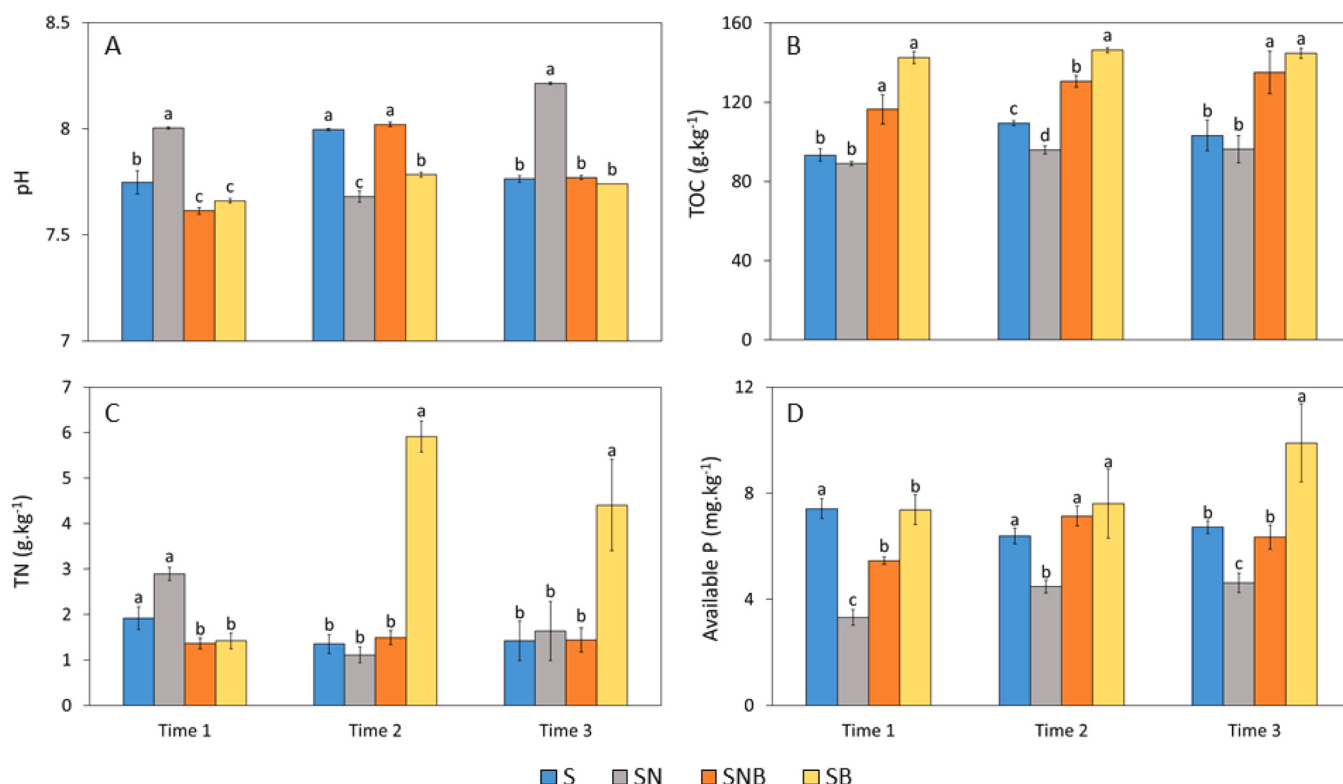


Fig. 1. Evolution of pH (a), TOC (b), TN (c), and available P (d) during the experiment. Different letters in different samples indicate significant differences (n = 3, ANOVA; P < 0.05).

goethite (α -FeOOH), magnetite (Fe_3O_4), and potentially maghemite (γ - Fe_2O_3). In particular, the detection of goethite, typically formed under moist and oxidative conditions, suggests advanced oxidation of nZVI over time. These mineralogical changes are consistent with the observed variations in soil pH and redox potential (Eh), further confirming the dynamic transformation of Fe^0 and its influence on the geochemical environment.

In contrast, the application of biochar led to a temporary decrease in soil pH at days 0 and 30 compared to the control, potentially due to the presence of acidic functional groups or the release of organic acids during early stages of interaction with the soil. However, by day 60, pH values in the biochar treatment were indistinguishable from those of the control (Fig. 1A).

The TOC results aligned with expectations, as variations in this parameter were solely attributed to the amount of carbon added, which occurred exclusively in treatments involving biochar. Consequently, soil in the SNB and SB treatments exhibited the highest TOC content at all tested time points (Fig. 1B). Notably, the biochar used had a TOC content of 742 g kg^{-1} , significantly higher than the 100 g kg^{-1} measured in the original polluted soil (Table 1). The application of biochar, whether alone or in combination with other amendments, typically increases soil carbon, particularly when the biochar contains a high C content due to the properties of the precursor material and the pyrolysis temperature (Forján et al., 2019; [44]). In turn, the application of nZVI alone did not affect the TOC content. In contrast, the SNB treatment resulted in an increase in this parameter (Fig. 1B), although not as pronounced as that observed with the sole application of biochar, likely due to interactions between nZVI and dissolved organic carbon [45].

Biochar application also caused a significant increase in TN content, mainly at 30 days (Fig. 1C), which is consistent with the findings of several authors who concluded that biochar made from plant biomass leads to an increase in TN and a reduction in N leaching [46]. The addition of nZVI to the soil did not result in a significant change in TN (Fig. 1C), even when applied simultaneously with biochar. This finding aligns with the observations reported by Zhu et al. [47], who highlighted the interaction and competition of nitrogen with other ions for adsorption sites in both biochar and soil. Such interactions may reduce the transfer of nitrogen from biochar to the soil, thereby mitigating potential nitrogen losses.

Moreover, the amendment of soil with nZVI caused a decrease in available P concentrations (Fig. 1D), which can be attributed to the strong affinity of nZVI for phosphate ions and its ability to form stable Fe-P precipitates, as demonstrated in previous studies [42,48,49]. A similar decrease in P availability was observed when nZVI was applied in combination with biochar, suggesting that the presence of nZVI governs the overall P immobilization process. In contrast, the sole application of biochar enhanced P availability to levels comparable to those of the untreated control (Fig. 1D), likely due to the increase in dissolved organic carbon and the abundance of negatively charged functional groups on the biochar surface, which can mobilize phosphorus through ligand exchange or complexation mechanisms [46,50,51]. These contrasting behaviors highlight the dominant role of nZVI in phosphorus immobilization and its interaction with co-applied organic amendments.

3.3. Analysis of cation exchange capacity (CEC)

In all cases (control soil and treatments), the CEC was above $25 \text{ cmol}_{(+)}\text{kg}^{-1}$ (Table 2), which is the usual threshold to identify CEC deficiencies [52,53]; i.e. conditions hindering the development of a stable vegetation cover could occur as a result of the low capacity of the soil to retain cations and thus provide nutrients to plants [54]. In this pilot experiment, the combination of nZVI and biochar did not increase the CEC compared to the control polluted soil. This observation could be attributed to the interaction between biochar and the Na^+ introduced by nZVI, resulting in Na^+ retention within the biochar rather than in the

Table 2

Concentrations of exchange cations (Ca^{2+} , K^+ , Na^+ , Mg^{2+} , and Al^{3+}) and cation exchange capacity (CEC) ($\text{cmol}_{(+)}\text{kg}^{-1}$) in the untreated control soil and treated soils.

	S	SN	SNB	SB
Time 1	Ca^{2+}	$32.88 \pm 2.49\text{a}$	$35.00 \pm 2.70\text{a}$	$30.40 \pm 2.97\text{a}$
	K^+	$1.39 \pm 0.06\text{c}$	$1.97 \pm 0.25\text{ab}$	$2.15 \pm 0.14\text{a}$
	Na^+	$0.79 \pm 0.22\text{b}$	$9.50 \pm 0.77\text{a}$	$3.74 \pm 0.23\text{a}$
	Mg^{2+}	$1.36 \pm 0.10\text{a}$	$1.44 \pm 0.10\text{a}$	$1.30 \pm 0.05\text{a}$
	Al^{3+}	$2.44 \pm 0.18\text{a}$	$1.79 \pm 0.81\text{b}$	$1.69 \pm 0.47\text{b}$
	CEC	$38.88 \pm 2.62\text{ab}$	$49.72 \pm 3.91\text{a}$	$39.30 \pm 2.97\text{a}$
Time 2	Ca^{2+}	$30.76 \pm 0.39\text{a}$	$34.18 \pm 1.94\text{a}$	$29.40 \pm 2.28\text{a}$
	K^+	$1.09 \pm 0.52\text{b}$	$1.95 \pm 0.07\text{a}$	$1.76 \pm 0.12\text{a}$
	Na^+	$0.60 \pm 0.13\text{b}$	$5.91 \pm 0.38\text{a}$	$2.55 \pm 0.13\text{a}$
	Mg^{2+}	$1.04 \pm 0.50\text{a}$	$1.48 \pm 0.11\text{a}$	$1.24 \pm 0.06\text{a}$
	Al^{3+}	$2.30 \pm 1.18\text{a}$	$2.06 \pm 0.79\text{a}$	$1.65 \pm 0.36\text{a}$
	CEC	$29.14 \pm 13.70\text{b}$	$45.60 \pm 2.59\text{a}$	$36.62 \pm 2.42\text{ab}$
Time 3	Ca^{2+}	$29.03 \pm 0.17\text{ab}$	$33.47 \pm 2.81\text{a}$	$28.46 \pm 3.68\text{b}$
	K^+	$0.92 \pm 0.03\text{b}$	$1.14 \pm 0.13\text{b}$	$1.54 \pm 0.30\text{a}$
	Na^+	$0.38 \pm 0.04\text{c}$	$2.81 \pm 0.35\text{ab}$	$3.36 \pm 0.06\text{a}$
	Mg^{2+}	$1.11 \pm 0.06\text{a}$	$1.54 \pm 0.17\text{a}$	$1.24 \pm 0.21\text{a}$
	Al^{3+}	$2.07 \pm 0.13\text{b}$	$2.12 \pm 0.28\text{b}$	$2.66 \pm 0.39\text{ab}$
	CEC	$33.53 \pm 0.23\text{b}$	$41.09 \pm 3.20\text{a}$	$35.27 \pm 5.50\text{b}$

For each row, different lowercase letters in the diverse samples signify significant differences between treatments ($n = 3$, ANOVA; $P < 0.05$). Units: $\text{cmol}_{(+)}\text{mg}^{-1}$.

soil due to its adsorption capacity [55]. Ca^{2+} was the cation with the highest values in both control and treated soils. In this regard, such high Ca^{2+} concentrations could derive from the slag present in the brownfield soil [3]. In addition, these high Ca^{2+} contents may explain the alkaline pH of the soil, given that the percentage of Ca^{2+} accounted for approximately 80.74 % of the CEC. However, despite the predominance of Ca^{2+} as the most abundant cation, the concentrations of the other cations were not limiting for plant growth [52].

3.4. Analysis of As, Cd, Cu, Pb and Zn availability in the soil

The effectiveness of the untreated amendments at immobilizing metals in the soil was evidenced by the decrease in available concentrations of Cu, Cd, Zn and Pb (Figs. 2A, 2B, 2C, 2D). This reduction was observed mainly at 30 and 60 days compared to the control soil. At the end of the experiment (60 days), the soils amended with biochar alone or in combination with nZVI showed the highest reductions in available Cu, reaching over 70 % (Fig. 2A). The capacity of biochar to immobilize Cu in the soil is primarily attributed to its ability to enhance the sorption capacity of metals in soil. This effectiveness is linked to factors such as the presence of organic matter, a large surface area, and functional groups such as $-\text{COOH}$, $-\text{CO}-$, $-\text{OH}$, and $\text{R}-\text{COO}-\text{R}$ [56,57]. Similarly, nZVI treatment also facilitated Cu immobilization, particularly at 30 and 60 days. As highlighted by Danila et al. [58], the effectiveness of nZVI in reducing Cu availability—a capacity attributed to Cu binding with Fe oxyhydroxides in an alkaline pH induced by nZVI oxidation. However,

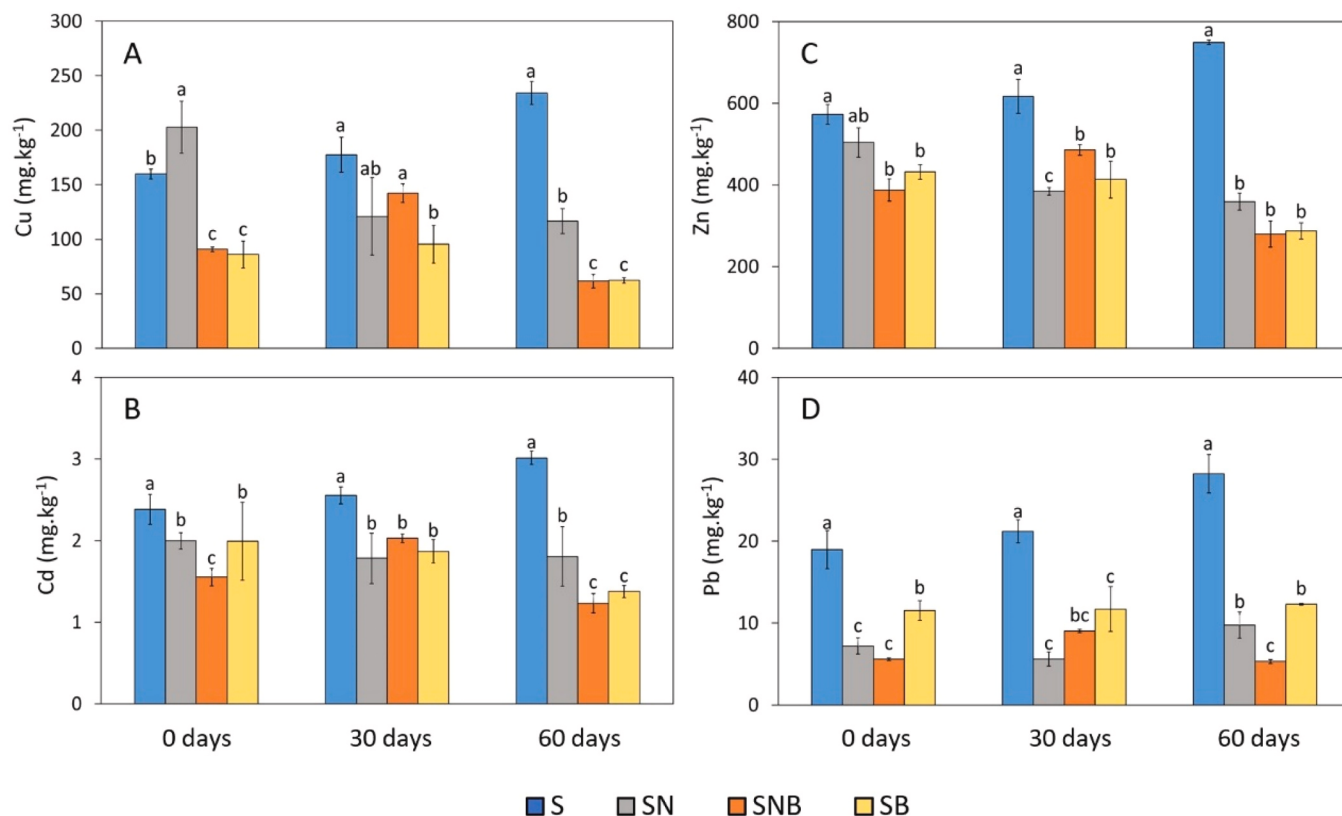


Fig. 2. Available Cu (a), Cd (b), Zn (c) and Pb (d) concentrations throughout 60-day experiment in control and treated soil. Different letters in different samples indicate significant differences ($n = 3$, ANOVA; $P < 0.05$).

initially, the nZVI amendment substantially increased Cu availability, possibly due to the presence of this metal in the nZVI (data not shown) or oxidation-reduction changes induced by the amendment. As mentioned previously, the combination of nZVI and biochar mitigated Cu mobilization caused by nZVI alone, thus revealing an advantage of this combined treatment.

The available concentration of Cd in the polluted soil was significantly lower compared to that of other metals. However, given its non-essential nature, high potential toxicity, and relative mobility in the soil-plant system, Cd cannot be overlooked [59]. In this regard, the treatments markedly reduced the availability of this pollutant in the soil. This effect was most marked when biochar was included (Fig. 2B), yielding a decrease of 59 % and 54 % at 60 days in the SNB and SB treatments, respectively, whereas the application of nZVI alone reduced Cd availability by 40.1 % at the same time point. Several studies [24,60,74] have demonstrated that biochar immobilizes Cd in soil primarily through sorption. In contrast, the immobilization mechanism of nZVI involves sorption or formation of surface complexes of Cd on Fe-oxides generated during nZVI oxidation in the soil [12,62].

Available Zn decreased mainly at 30 and 60 days, reaching final reductions of 43 %, 62 %, and 61 % in the SN, SNB, and SB treatments, respectively. No statistical differences were found between the treatments at the end of the experiment (Fig. 2C). In this regard, biochar application alone or in combination with nZVI proved to be the most effective strategy to immobilize Zn. The application of nZVI also emerged as less efficient than biochar, revealing a decrease of 12 % vs. 24 % when using biochar at the beginning of the experiment. Zn immobilization by nZVI is attributed to the fact that this metal has a lower standard potential (E^0) than elemental Fe (-0.41 V), and, as a result, immobilization occurs exclusively through sorption, following a surface complexation mechanism [62,63]. As reported previously for the other metals, soil amendment with biochar, which has a high specific

surface area and is negatively charged with functional groups, favours Zn immobilization (Forjan et al., 2016a). In fact, Beesley et al. [64] concluded that sorption is the main mechanism by which biochar reduces Zn mobility in soils.

The amendments proved especially useful for immobilizing soil Pb, the best results being achieved in the treatments that involved nZVI alone or combined with biochar (Fig. 2D). At the end of the experiment, the nZVI-biochar tandem resulted in an 82 % decrease in Pb mobility, revealing a synergistic effect between the amendments as the sole application of nZVI or biochar led to a 65 % and 56 % decrease in this parameter, respectively. The precipitation and transformation of hydrated Pb/Fe oxides in soil particles may also explain the decrease in available Pb concentrations caused by nZVI application [65]. On the other hand, biochar can reduce the availability of Pb in the soil due to the increase in the CEC and its specific surface area [60,66]. These two factors contribute to the capacity of biochar to reduce the availability of metals through the complexing of metal ions on its surface [56]. Additionally, biochar reduces Pb mobility by precipitating insoluble Pb phosphates [61], as the amount of P present in the biochar was higher than that in the polluted soil (Table 1), which was observed as an increase in soil P content after the application of this amendment (Fig. 1D).

With respect to As, the availability of this metalloid was not properly assessed using TCLP extraction due to the limited detection capacity of the technique. However, the effects of nZVI on As immobilization are discussed below from the perspective of As accumulation in plants. Both nZVI and biochar can influence arsenic mobility through complementary redox and sorption mechanisms, and their combined application may enhance immobilization efficiency by promoting adsorption, coprecipitation, and nanoparticle stabilization [11,24].

3.5. Growth and physiological parameters of *Brassica juncea* L

3.5.1. Growth and mineral nutrient accumulation in plants

After 60 days of cultivation, the plants grown in soil treated with biochar (SB) showed more biomass, measured as fresh and dry weight, than those grown in the untreated polluted soil (S) (Table 3). This finding could be attributed to the release of nutrients from the biochar into the soil, enhancing plant growth and development [7,67]. Additionally, the ability of biochar to increase water field capacity in soil likely contributes to improved water retention, resulting in a greater amount of water available to plants, which in turn is reflected in increased biomass production [7,68]. This greater growth is also supported by a better nutritional balance in plants grown in SB since a higher concentration of Ca, K, Mg and P was observed when compared to those grown in S (Table 3). These findings are consistent with previous studies by Baragaño et al. [5] and Rodríguez-Vila et al. [69], which demonstrated the positive impact of organic amendments on various soil parameters, leading to enhanced plant growth. The addition of nZVI (SN) resulted in a significant decrease in biomass compared to plants grown in untreated polluted soil (S) (Table 3) as also observed by Huang et al. [70] and Zand and Tabrizi [71], who concluded that an excessive dose of nZVI can impede biomass development and could potentially impair nutrient uptake, as indicated by the nutrient analysis conducted herein (Table 3). In our case, this effect was not observed, although an improvement in the CEC of the soil was detected (Table 2), which is primarily attribute to an increase in Na^+ . However, in plants, a reduction in the content of the analyzed nutrients was observed (Table 3). Although the dose of nZVI used in this study was not excessively high, it still impacted biomass, root length, and the concentration of nutrients (Table 3). When nZVI and biochar were applied together to the soil (SNB), thereby diluting the potential excess of nZVI, biomass increased compared to SN, and the concentration of nutrients was higher than in SN but lower than in SB (Table 3). Similar results were also reported by Zand et al. [7], who found that the application of biochar (5 %) in combination with nZVI resulted in higher harvested biomass compared to that achieved when only nZVI were added to the soil possibly.

3.5.2. Accumulation of metal(oid)s in the plant

Metal(oid) accumulation was higher in the roots than in the aerial part of the plants (Fig. 3). This observation is logical considering that the root is the first organ of the plant in contact with the soil and serves as the primary organ for the absorption of both essential and toxic elements. To prevent toxicity to plant metabolism, toxic metal(oid)s are rapidly stored in root cell vacuoles or are adsorbed to the root cell walls without entering the cells themselves [39,72,73].

Plants grown in nZVI-amended soil (SN) exhibited a decrease in the accumulation of As, Cd, Cu, Pb and Zn in the roots compared to plants

grown in untreated soil (S). In the aerial part, the concentrations of As and Pb decreased, whereas, unexpectedly, the Zn concentration increased. When biochar was added (SB), lower accumulation values were obtained for all metal(loid)s, except for As in aerial part (Fig. 3). Co-application of nZVI and biochar (SNB) reduced Cd, Cu, Pb and Zn uptake by the roots and increased As accumulation in shoots compared with the treatments SN. This finding is attributed to the high adsorption capacity of this material, as well as its specific surface area, and the presence of carboxyl, amide, and CH_2 groups, which facilitate the immobilization of metals in soil [57,74].

The total As concentration in the soil exceeds the thresholds established by current legislation. However, the mobility of As could not be determined using the applied methodology, as the As concentration in the extract was below the detection limit of the equipment used. It is important to note that As mobility may vary over time due to a range of factors, including those associated with global and climate changes [75]. In this regard, the monitoring of this metalloid in plants has been evaluated, revealing low As concentrations. Nevertheless, under long-term conditions or alternative scenarios, these concentrations could potentially reach levels indicative of toxicity for the plants ($1\text{--}20 \text{ mg.kg}^{-1}$ according to [76]). Additionally, As concentrations in plants were not consistent across all treatments analyzed (Fig. 3B). Plants grown in SN showed a decrease in As accumulation in the aerial part, which is consistent with expectations based on previous studies [15]. Briefly, nZVI acts through redox-mediated immobilization, reducing As(V) to As(III) and promoting the formation of poorly soluble Fe-As precipitates (e.g., scorodite or ferric arsenate), or facilitating adsorption onto iron oxides formed as nZVI oxidizes (Baragaño et al., 2020d).

Conversely, those in the biochar treatment (SB) showed an increase in As accumulation in these parts (Fig. 3B). This observation is consistent with reports in the literature, since As is mobilized in soils due to the interaction of the negatively charged surface of biochar, thereby favouring the uptake of this element by plants (Beesley et al., 2011a). Other authors [68,77] have also observed that soil amendment with biochar increases the concentration of As and P in the plant (as in our case in the aerial part). When applied in combination, biochar can stabilize nZVI nanoparticles, reducing aggregation and enhancing their reactivity, while also acting as a secondary sorbent phase. This combined system can generate a more reducing and alkaline microenvironment, thereby promoting As immobilization through enhanced adsorption and co-precipitation processes. As a result of this dual mechanism, As phytoavailability is significantly reduced, limiting its solubility and uptake by plants. Consequently, As accumulation in plants under the combined treatment was higher than in the SN treatment, but lower than in the SB condition (Fig. 3B).

Plants grown in SN showed a decrease in the accumulation in Zn accumulation in the roots; however, a surprisingly increase was observed in the aerial part of *Brassica juncea*. This could be attributed to the fact that Zn is an essential micronutrient required for plant growth, playing a crucial role in redox reactions and serving as an enzymatic cofactor in numerous metabolic pathways [78]. In contrast, other toxic elements, such as As, Cd, and Pb, have no known function in plants, and their absorption occurs as a result of the similarity of their chemical characteristics to other essential elements such as Ca, P, Fe, or Zn [79].

The translocation of metal(loid)s from roots to shoots and their accumulation in shoots is an important index for evaluating the phytoextraction potential of plants. In this study, TF values for the analyzed metal(loid)s increased in all amended treatments compared to the polluted soil (S) (Table S4). Literature suggests that the application of high doses of nZVI may inhibit the translocation of metal(loid)s in plants due to the depletion of soil oxygen caused by the oxidation of the nanoparticles [70,80]. However, in this work it was observed an increase in TF across all cases, likely because the concentrations of nanoparticles applied to the soil were not high enough to inhibit this translocation.

Table 3

Growth parameters and mineral nutrient accumulation in the aerial part of *Brassica juncea* L. after 60 days of growing in the polluted control soil and treated soil.

Parameter	Units	S	SN	SNB	SB
Fresh Biomass	g.m^{-2}	457 $\pm$ 17b	230 $\pm$ 23c	396 $\pm$ 17b	658 $\pm$ 24a
Dry biomass	g.m^{-2}	65 $\pm$ 6b	44 $\pm$ 3c	59 $\pm$ 3b	96 $\pm$ 2a
Height		52 $\pm$ 3a	32 $\pm$ 9b	27 $\pm$ 6b	42 $\pm$ 4ab
Root length	cm	11.9 $\pm$ 1.1a	8.5 $\pm$ 1.5a	9.8 $\pm$ 1.9a	9.7 $\pm$ 1.7a
Ca		1413 $\pm$ 2b	505 $\pm$ 1d	917 $\pm$ 1c	1965 $\pm$ 1a
K	mg.kg^{-1}	2409 $\pm$ 1b	863 $\pm$ 1d	1853 $\pm$ 2c	3341 $\pm$ 1a
Mg		137 $\pm$ 1b	51 $\pm$ 2d	102 $\pm$ 1c	239 $\pm$ 1a
P		187 $\pm$ 1b	96 $\pm$ 2d	140 $\pm$ 2c	300 $\pm$ 1a

For each row, different lowercase letters in the different samples signify significant differences between treatments (n = 3, ANOVA; P < 0.05).

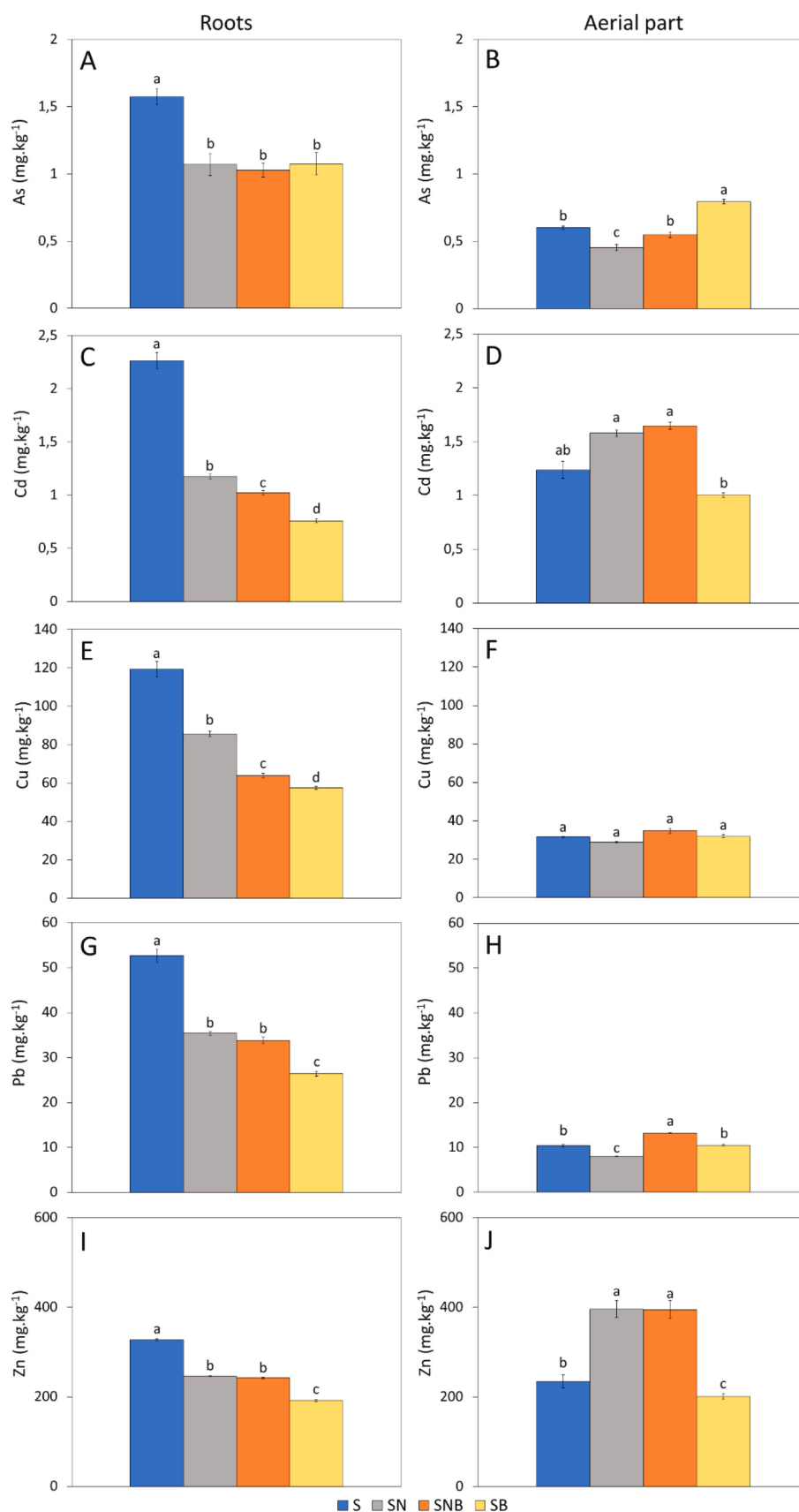


Fig. 3. Accumulation of metals in roots (left) and aerial part (right) of *Brassica juncea* L. after 60 days of growth in the polluted control soil and treated soil. Different letters in different samples indicate significant differences ($n = 3$, ANOVA; $P < 0.05$).

The TF for As significantly increased after biochar application, which could explain the higher As concentration in plants in the SB treatment than in S or SN. It is difficult to determine why the plants in the SB treatment accumulated less As in the root but doubled the TF, leading to a greater accumulation of this element in the aerial part. This observation could be attributed to the greater growth (measured as fresh weight or dry weight) of plants in soil amended with biochar and therefore the greater demand for P to support the development of buds and leaves. As P and As are chemically analogous, a greater amount of As would also reach the aerial part of the plants. The fact that As can substitute P inside the cell but cannot carry out the same metabolic function could be perceived by the plant as P deficiency, and P uptake might increase [81], as also observed in our case. For Cu and Pb, the TF values increased with all amendments, although the values remained below 1. The most significant changes were observed for Cd and Zn, with TF values exceeding 1. The increase in TF for Zn was particularly notable in treatments involving nZVI (SN and SNB), while for Cd, a synergistic effect between nZVI and biochar was observed, resulting in the highest TF value for this treatment. Interestingly, the co-application of nZVI and Biochar produced the highest TF values reaching the value of 1 for As and exceeding the value of 2 for Zn and Cd (Table S4), indicating that the simultaneous application of both amendments stimulates the metal(lod)s translocation in plants. TF values higher than 1 for Zn have also been reported in other studies using *Betula pubescens* and *B. juncea* L. [39,82].

3.5.3. Photosynthetic pigments and photosynthetic efficiency

The results shown in Table 4 reveal that the highest photosynthesis values occurred in plants grown in the polluted control soil (S) while the lowest were detected in the plants cultured in the SB and SNB treatments. Plants growing under metal(lod)s stress often present photosynthesis inhibition, as these elements can be highly toxic and disrupt key metabolic processes, ultimately impairing the photosynthesis [39, 81,83]. In our case, the application of nZVI to the soil resulted in a reduction in photosynthesis, consistent with findings reported by other authors [84,85]. This phenomenon can be attributed to the potential of nZVI to alter the physiology of membranes and disrupt the balance of enzymes and organelles, ultimately affecting the photosynthetic activity. The particularly low photosynthesis value observed in the SB treatment may be explained by the higher concentration of As detected in the leaves, as this metalloid can disrupt the electron transport chain, thereby affecting the ultrastructure of the chloroplast and the photosystems [68]. The lowest photosynthesis values were observed in plants with high metal accumulation in the aerial part, as corroborated by the significant negative correlation between photosynthesis values and metal concentrations in the aerial part (-0.87 and -0.92 for Zn and Cd, respectively, $P < 0.01$). The transpiration values were higher in the plants grown in S than in the other treatments (Table 4). The decrease in transpiration in plants cultured in SB may be explained by the fact that this material retains a greater amount of water in the soil, which would result in a lower amount of water available to the plant, thus reducing its absorption and therefore its transpiration [86]. The decrease in transpiration in plants in the SN treatment (Table 4) could be attributed to the capacity of nZVI to compact the soil, thereby hindering the uptake of

water and root growth (Table 3). When stomatal conductance was measured, the profile was similar to that of transpiration (Table 4). Of note, a greater transpiration rate of *B. juncea* L. was related to high metal accumulation especially in the root as both metal accumulation and transpiration were higher in plants grown in the control soil.

Analysis of the photosynthetic pigments content revealed that plants grown in SB treatment exhibited the highest values of Chl a, Chl b and carotenoids. However, these values did not correspond to the highest photosynthesis rates (Table 4) but were associated with the best growth outcomes, as reflected in FW and DW. This trend also correlated with the highest concentration of Mg in the aerial part of the plants. The Mg^{2+} is a relevant component of the chlorophyll molecule and plays a fundamental role in photosynthetic processes [87]. Interestingly, it should be noted that this treatment showed the lowest photosynthesis values; possibly, due to As accumulation in the leaves, which may have inhibited photosynthesis. However, the As concentration was not high enough to impede the synthesis of pigments. In plants of SN treatment, the Chl a, b and carotenoids content was the lowest, which could explain the presence of chlorosis in the leaves (data not shown). Furthermore, some authors propose that this reduction in chlorophyll content may be due to the fact that nZVI can affect biochemical factors such as lipid peroxidation in photosynthetic membranes, although in our case it was not observed (Fig. 4). On the other hand, given the high concentration of Zn in the aerial part of plants grown in SN treatments, this element could replace or even inhibit the function of Fe or Mg^{2+} [88].

3.5.4. Oxidative stress analysis: hydrogen peroxide (H_2O_2), lipid peroxidation (MDA) and proline

Leaves showed higher contents of H_2O_2 than the roots (Fig. 4A). In this organ, no differences were observed between the treatments while the leaves of SN treatment showed the highest H_2O_2 content and those in the SB condition showed the lowest (Fig. 4A). This decrease observed in SB may be linked to increased plant growth (significant negative correlations, 0.87, $P < 0.01$) and the protective role of biochar under biotic and abiotic stress conditions [68], potentially due to increased activity of certain antioxidant enzymes (catalase and superoxide dismutase) [89], though enzyme activity was not evaluated in this study. Conversely, the elevated H_2O_2 content in leaves of plants grown in nZVI-amended soil could result from decreased antioxidant enzyme activity as has already been hypothesized by Huang et al. [90].

In leaves, the lowest MDA content also occurred in plants in the SB treatment while in roots the values were lower than in the control soil (S). In this context, significant positive correlations were observed between the Cd and Zn content in the aerial part and the MDA content (0.98 and 0.94 respectively, $P < 0.01$). Hafez et al. [91] and Hasanuzzaman et al. [83] highlight that biochar can significantly protect the stability of the plasma membrane to stress, regulating the water content of plants and therefore reducing oxidative stress and lipid peroxidation. Again, it seems that the addition of nZVI to the soil causes higher leaf stress, as reflected by an increase of more than 60 % in the content of MDA compared to the leaves of plants in the biochar treatment. Ghnaya et al. [92] proposed a relationship between the concentration of metals in leaves and the increase in MDA, which was also observed in our SN

Table 4

Photosynthesis, transpiration, stomatal conductance, and photosynthetic pigments of *Brassica juncea* L. after 60 days of growing in the polluted control soil and treated soil.

Parameters	S	SN	SNB	SB
Photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	14.1 ± 0.5a	7.9 ± 0.3b	6.7 ± 0.1c	6.8 ± 0.1c
Transpiration ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	4.5 ± 0.2a	3.7 ± 0.1b	3.8 ± 0.1b	3.9 ± 0.1b
Conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	0.2 ± 0.0a	0.1 ± 0.1c	0.1 ± 0.1c	0.1 ± 0.0c
Chlorophyll a ($\mu\text{g g}^{-1}$ PF)	286.7 ± 69.8b	203.8 ± 35.1c	375.1 ± 57.3b	411.7 ± 87.3a
Chlorophyll b ($\mu\text{g g}^{-1}$ PF)	101.1 ± 15.5b	75.3 ± 11.1b	86.3 ± 8.4b	152.5 ± 24.2a
Chlorophyll a+b ($\mu\text{g g}^{-1}$ PF)	387.8 ± 85.1b	279.1 ± 46.2c	461.4 ± 65.5ab	564.2 ± 64.0a
Carotenoids ($\mu\text{g g}^{-1}$ PF)	85.3 ± 3.9b	82.2 ± 10.3b	144.9 ± 6.5a	139.5 ± 14.3a

For each row, different lowercase letters in the different samples signify significant differences between treatments ($n = 3$, ANOVA; $P < 0.05$).

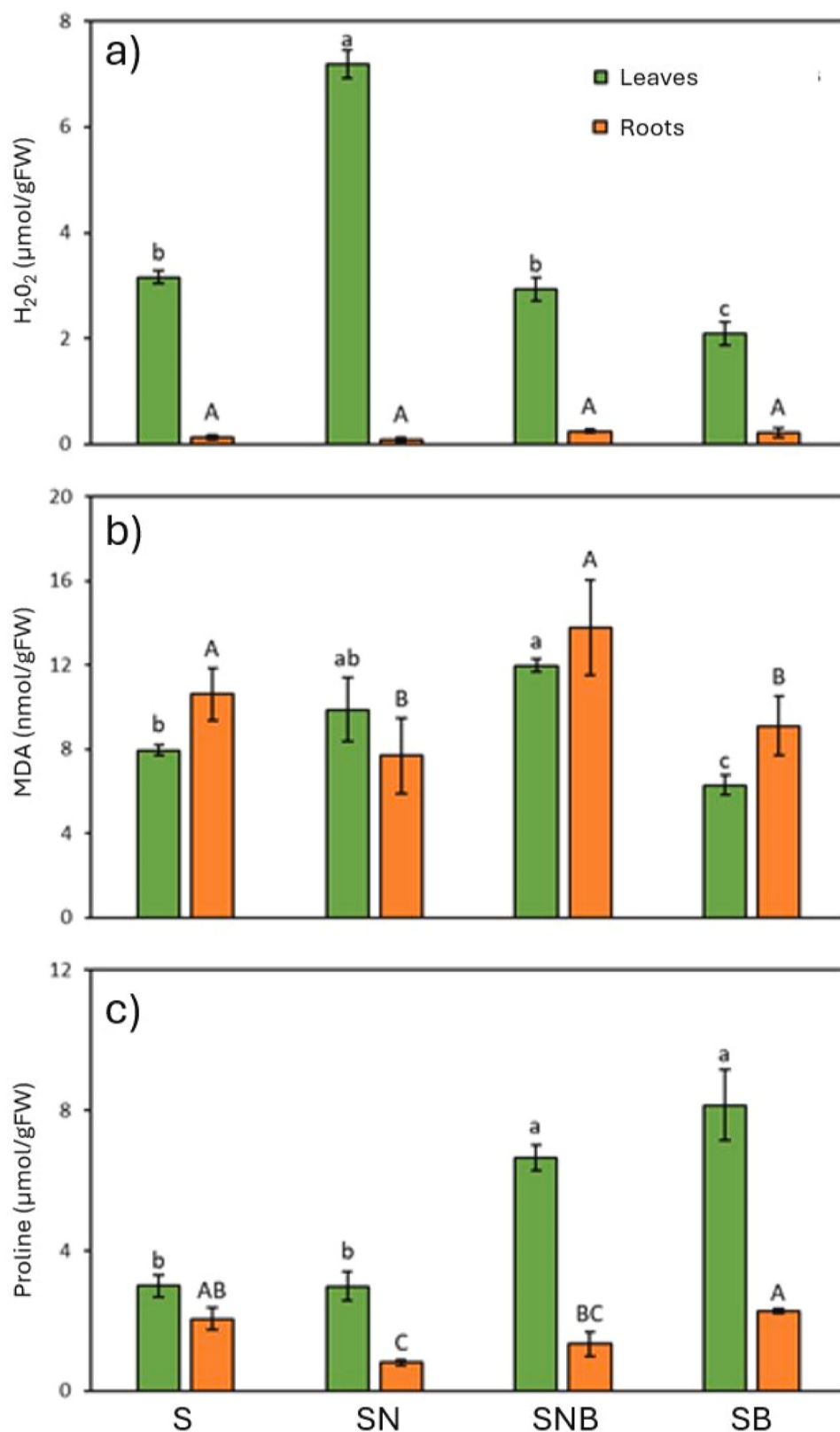


Fig. 4. Concentrations of hydrogen peroxide (A), lipid peroxidation (B), and proline (C) in the leaves and roots of *Brassica juncea* L. after 60 days of growth in polluted control soil and treated soil. Different lowercase letters indicate significant differences in leaves, while uppercase letters indicate significant differences in roots.

treatment and would explain the decrease in the content of chlorophyll a and b, leading to an increase in chloroplast membrane peroxidation.

Regarding proline, a higher content was observed in leaves than in

roots (Fig. 4C). This finding is consistent with the results reported by Sharma and Dietz [93]. The leaves of plants grown in SB showed the highest proline content, while the plants in the S and SN treatments

showed the lowest (Fig. 4C). In roots, the lowest proline content was registered in SN treatment (Fig. 4C). The increase in the proline content in plants under the SB treatment could be attributed to the multifunctional role of this amino acid. Beyond its function as an osmotic regulator, proline contributes to the stabilization of membranes, acts as a reactive oxygen species (ROS) scavenger, protects enzymes, and serves as an As chelator, thereby aiding plants in recovering from stress [38, 68]. This relationship is further evidenced by the significant statistical correlation observed between proline content and As accumulation in the plant ($0.85, P < 0.01$). Moreover, these findings align with Kamran et al. [68], who reported that biochar enhances plant nutritional status and promotes proline production, thereby mitigating stress effects.

4. Conclusions

Soil contamination by arsenic and other metals remains a critical environmental challenge, particularly under field conditions where complex contaminant interactions and site-specific variability limit the effectiveness of conventional remediation strategies. This study provides field-based evidence supporting the potential of biochar and nZVI as complementary amendments for the stabilization of multi-element contaminated soils. When applied individually, both materials reduced metal(loid) mobility, with the combined application showing a synergistic effect, particularly in enhancing Pb immobilization. These findings suggest that amendment combinations can be strategically designed to target specific contaminants more effectively.

Importantly, the study also reveals the contrasting effects of the amendments on plant health. While nZVI alone induced phytotoxic effects and oxidative stress in *Brassica juncea*, biochar mitigated arsenic-induced toxicity, improved biomass production, and contributed to oxidative stress alleviation. The co-application of both amendments not only enhanced contaminant immobilization but also reduced the negative impacts of nZVI on plant physiology, underscoring the importance of balancing chemical stabilization efficacy with plant compatibility in phytomanagement approaches.

By integrating contaminant dynamics, soil properties, and plant physiological responses, this work demonstrates the feasibility and added value of hybrid strategies that combine chemical and biological remediation mechanisms under real-world conditions. These insights are particularly relevant for the development of scalable, site-specific remediation frameworks. Future research should focus on long-term amendment stability, repeated planting cycles, and broader ecological impacts, including microbial community responses, to ensure the sustainability and resilience of such integrated approaches.

CRedit authorship contribution statement

González: Writing – review & editing, Supervision, Formal analysis, Conceptualization. **José Luis R Gallego:** Writing – review & editing, Funding acquisition. **Rubén Forján:** Writing – review & editing, Formal analysis. **Isabel Suárez:** Writing – review & editing, Formal analysis, Data curation. **Diego Baragaño:** Writing – original draft, Supervision, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2025.117607.

Data availability

Data will be made available on request.

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