# The influence of nZVI composites as immobilizing agents on the bioavailability of heavy metals and plant growth in mining metal-contaminated soil

**Mohammed Awad Mousa***, **Abdullah S.F. Al-Farraj, Mohammad I. Al-Wabel, Hesham M. Ibrahim, Adel R. A. Usman, Hamed A. Al-Swadi, Munir Ahmad***, **and Mohammad Imran Rafique**

Department of Soil Science, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh, 11451, Saudi Arabia, 441106289@student.ksu.edu.sa; sfarraj@ksu.edu.sa; malwabel@ksu.edu.sa; habdo@ksu.edu.sa; adosman@ksu.edu.sa; halswadi@ksu.edu.sa; amunir@ksu.edu.sa; mrafique@ksu.edu.sa.

*Corresponding author e-mail: 441106289@student.ksu.edu.sa, tel: 00966537224700; amunir@ksu.edu.sa, tel:00966542335725.

**Graphical Abstract**

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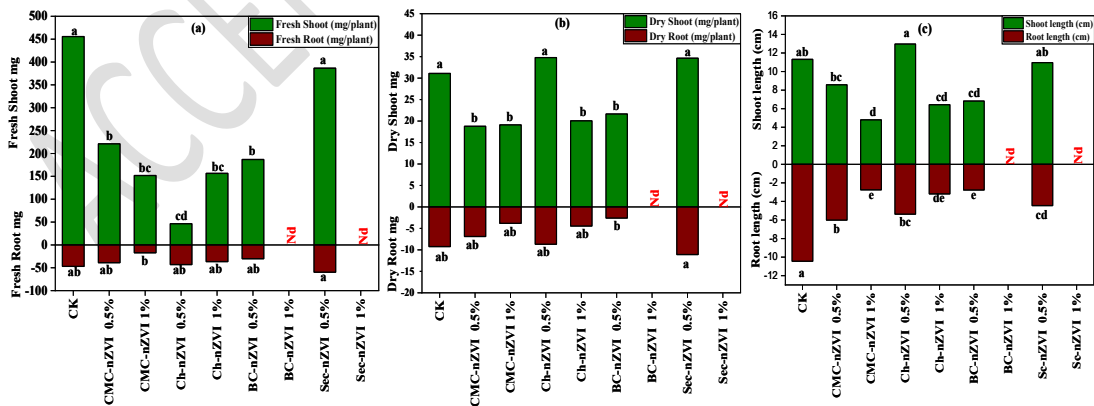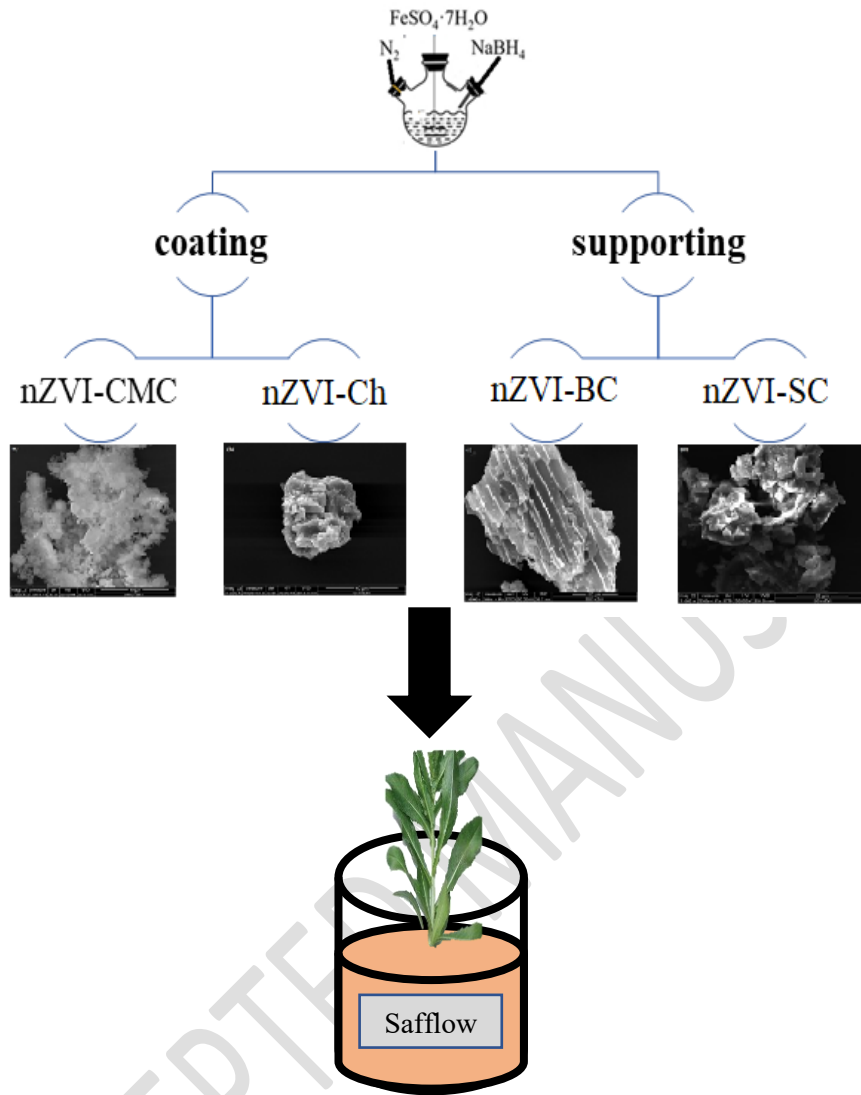
**Abstract**

Under the greenhouse scale, nano zero valent iron (nZVI) have been evaluated evaluated 18
by formulation with carboxymethyl cellulose (CMC-nZVI), chitosan (Ch-nZVI), biochar 19
(BC-nZVI), and smectite (SC-nZVI) at rates of 0 and 0.5 and 1% (w/w). The properties of 20
manufactured compounds and their impact on the soil ecosystem planted with safflower 21
(Carthamus tinctorius L.) as a phytoremediation crop for inorganic contaminants in 22
contaminated mining soils have been measured. After 33 days, growth parameters were 23
recorded in harvested plants and the concentrations of some HMs in plant tissues. 24
Furthermore, pH, EC, soluble Na and available Cd, Cu, Pb and Zn concentrations in the 25
treated soil after harvest were analyzed. Results revealed that Ch-nZVI and SC-nZVI 26
application at 0.5% led to a narrow non-significant increase in the dry biomass and the 27
shoot length compared to the control. On the other hand, the application of CMC-nZVI and 28
BC-nZVI resulted in reduced plant growth. All the applied amendments increased soil pH, 29
EC, and soluble Na contents. The soils treated with synthesized nZVI composites showed 30
a considerable increase (non-significant) in available heavy metals in treated soils 31
compared to control. Application of CMC-nZVI1% resulted in increased shoot copper (Cu) 32
and zinc (Zn) (182% and 114%, respectively, compared to control), while Ch-nZVI, SC- 33
nZVI applied at 1%, and BC-nZVI0 applied at 5% resulted in reduce Cu, Zn, and Pb 34
concentrations in roots by 65.2%, 31.4% and 85.5% in Cu, Zn and Pb respectively as 35
compared to control). The phytoremediation indices showed that the uptake and extraction 36
of HMs by safflower plants weren't significant in decreasing heavy metals in polluted soil 37
and the applied materials disabled the actions of hyperaccumulated plant used. Results 38
showed that these materials didn't have a significant effect on immobilize of HMs in 39
mining soil, while the application of Ch-nZVI, SC-nZVI, and BC-nZVI resulted in disabled 40
hyperaccumulated plant to reducing the uptake of Pb, Cu, and Zn. It could be concluded 41
that the application of synthesized nZVI composites to mining polluted soil might be 42
restricted, mainly due to their negative impacts on soil ecosystem besides their side effects 43
on HMs uptake by test hyperaccumulated crop. 44

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**Keywords:** nZVI based composite materials, heavy metal, bioavailability, contaminated 46
mining soil, remediation 47

## 1. Introduction

Soil pollution with heavy metals (HMs) contaminants adversely affects the properties of soil, the yield of crops, and the health of humans. With the progress in agricultural and industrial development, soil and groundwater resources were polluted with enormous concentrations of HMs exceeding the standard limits. Heavy metals are persistent, non-biodegradable human pollutants that can deteriorate the environment and are hazardous for plants, animals, and humans (Al-Swadi *et al.* 2022). On Earth, natural sources of metalloids and HMs are present; however, anthropogenic activities have significantly increased the amount of HMs in the environment. Mining activity releases various harmful substances, such as liquid and solid waste. According to studies, Cd, Cu, Pb, and Zn are the primary contaminants detected in the soil in mining areas. These contaminants deteriorate the soil and have a negative effect on human health because they accumulate in the edible parts of crops (Jia et al. 2018). Therefore, mining soil remediation is of importance to reduce negative effect of toxic potential metals. The various in situ and ex situ techniques have been reported to remediated soil HMs (Yuan *et al.* 2017). Chemical stabilization is a characteristic in situ approach that effectively immobilizes metals by utilizing a variety of adsorbent materials (Mandal *et al.* 2020. The remediation of HMs pollution using adsorbent materials has been the focus of many studies because of their ease of application and high efficiency. Common adsorbents include clay materials, organic matter, biochar, and metal oxides (Mandal *et al.* 2020). nZVI is one of these materials that has drawn the most attention since it is a superior HMs adsorbent. The high surface area, abundance of reactive surface sites, and eco-friendly nature of nZVI have contributed to the high efficiency of nZVI when applied to remediate HMs contaminants from systems of groundwater and soil (Zhang *et al.* 2019). According to Gil-Diaz *et al*. (2017), applying 10%, nZVI boosted Pb, Cr, and Zn immobilization by more than 79%; however, nZVI application did not significantly improve Cd immobilization. For nZVI, the most common support materials include activated carbon, silica, montmorillonite, kaolinite, and biochar. Support materials reduce particle aggregation and increase the efficiency of nZVI in remediation processes (Wang *et al.* 2017). In another study, the adsorption capacity of

arsenate and arsenate on nZVI was significantly increased and when nZVI particles were 78
supported on activated carbon (Xu *et al*. 2014). A reverse trend was observed when the 79
investigation was conducted into the occurrence, characterization, measurement, and 80
environmental applications of (nZVI) (Jawed et al. 2023).In contras a thorough 81
investigation has been conducted into the occurrence, characterization, measurement, and 82
environmental applications of (nZVI) (Jawed et al. 2023). However, just a few papers have 83
briefly discussed nZVI regarding environmental uses, risk, fate, and soil contexts. The 84
majority of research centered on using nZVI chemicals in aquatic settings. For instance, 85
Petosa et al. (2010) evaluated the effectiveness of nanoparticles for the purification of water 86
and looked at the behaviors and toxicity of iron based in aquatic environments, while Lei 87
et al. (2018) investigated the aggregations and depositions of nZVI in aquatic 88
environments. The injection of nanoscale zero-valent iron (nZVI) into a saturated soil has 89
demonstrated several beneficial effects, as observed in a study conducted by Němeček et 90
al. (2014). The primary focus of the study was the immobilization of Cr(VI) in polluted 91
groundwater, and it was found that the injection of nZVI led to a rapid immobilization of 92
Cr(VI). Studies have reported that the use of nanoscale zero-valent iron (nZVI) supported 93
on different types of materials, such as biochar (BC-nZVI), can serve as a stable adsorbent 94
for the effective removal of various contaminants, including metals, from aqueous 95
solutions (Qian et al., 2019). Overall, the findings of the study by Hasan et al. (2020) 96
suggest that BC-nZVI is a promising and effective adsorbent for the removal of heavy 97
metals, specifically Cd and Zn, from synthetic stormwater, where the results showed BC- 98
nZVI demonstrated a 43% and 57% improvement in metal removal efficiency for Cd and 99
Zn, respectively. BC has been effectively used to eliminate pollutants from water and soil. 100
Considering the benefits and drawbacks of biochar and nZVI, it is highly possible that the 101
formation of BC-nZVI composite will help to minimize the limitations of both products, 102
and the combined features of nZVI-BC would be enhanced, leading to better remediation 103
efficiency (Yang *et al.* 2018). Natural clay deposits have also supported nZVI during 104
remediation processes (Bhowmick *et al*. 2014). 105

Though extensive studies of the occurrence, characterization, measurement, and 106
environmental applications of (nZVI) has been carried out in the last few decades (Jawed 107
et al. 2023), just a few papers have briefly discussed the using of nZVI in minimizing the 108

potential toxicity of inorganic pollutants in soil ecosystem and the growth of plants.
Therefore, this study was aimed to investigate the effects of synthesized nZVI composites
(CMC-nZVI, Ch-nZVI, BC-nZVI, and SC-nZVI) on immobilization of soil heavy metals
(Cd, Cu, Pb, Zn) and the growth parameters of safflower (*Carthamus tinctorius* L)
hyperaccumulator plant.

## 2. Materials and methods

2.1 *Soil sampling and analysis*

 The polluted samples of soil were collected from the mining region of Mahad AD'Dahab
region, Saudi Arabia ($23^{\circ}$ $29'$ $59.88''$ N and $40^{\circ}$ $51'$ $56.72''$ E). The soil samples were
collected at a 0–30 cm depth and combined to form a composite soil sample. After carefully
mixing, all soil samples were air dried, and then the soil samples were sieved using a 2 mm
screen. The soil sample was prepared for HMs and routine analyses with three replications.
In saturated soil paste, the pH of the soil was determined, and EC was analyzed in its
extracts (Aboukila and Norton 2017). The hydrometer method measured the soil texture
(Mwendwa 2022). The amount of calcium carbonate ($CaCO_3$) in the soil was ascertained
using the calcimeter (Erskine *et al.* 2017 ). The method described by Mylavarapu *et al.*
(2014) for determining soil organic matter (OM) involved potassium dichromate oxidation.
The total concentration of Cd, Cu, Pb, and Zn in the collected samples was determined
using the Hossner technique (total) (Hossner 1996). After cooling, the digested solutions
were filtered through Whatman 42 filter paper and transferred to a volumetric flask holding
50 mL of deionized water. AB-DTPA extraction method was used to measure the available
HMs in the soil (Hosseinpur *et al.* 2015). The concentrations of available and total HMs
were measured using an inductively coupled plasma-optical emission spectrometer (ICP-
OES: PerkinElmer Optima 4300 DV, USA). The results of the total concentrations of HMs
are presented in Table 1. The total amounts of Cd, Cu, Pb, and Zn in the soil were 18.81,
783.23, 329.87, and 3325.02 mg kg$^{-1}$, respectively. The soil's electrical conductivity value
was 1.05 dS m$^{-1}$, and its pH was slightly alkaline (7.48).

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**Table 1.** Some physiochemical characteristics and heavy metals concentrations of soil collected from Mahad AD'Dahab mining area.

| Soil property | Value |
|---|---|
| Particle size distribution | |
| Silt, % | 26.43 |
| Clay, % | 4.94 |
| Sand, % | 68.63 |
| Texture | sandy loam |
| EC | 1.05 |
| pH | 7.48 |
| Calcium carbonate, % | 7.23 |
| Organic matter, % | 1.9 |
| Soluble ions meq/L | |
| $Ca^{++}$ | 5 |
| $Mg^{++}$ | 3.6 |
| $Na^+$ | 1.7 |
| $K^+$ | 0.12 |
| $Cl^-$ | 7 |
| Total heavy metals content, mg/kg | |
| Zn | 3325.02 |
| Cu | 783.23 |
| Cd | 18.81 |
| Pb | 329.87 |

2.2 *Biochar production and smectite collection*

In this research, the biochar was prepared by pyrolysis of conocarpus (Conocarpus species) waste gathered from the campus of King Saud University, Riyadh, Saudi Arabia. Subsequently, the conocarpus waste underwent pyrolysis for three hours at $450^{\circ}C$; the resulting biochar was then ground and sieved through a 100 mesh (Al-Wabel *et al.* 2015), the biochar was grinded and sieved through 60 µm. The clay deposits were rich in smectite

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(> 85%) and were collected from the Khales region. The collected samples were grounded to pass a 60 µm sieve and subjected to physical (particle size distribution and total surface area).

## 2.3 *Synthesis of nZVI composites*

CMC-nZVI was prepared by using a tweaked variation of the procedure presented by Yan *et al.* (2013). Briefly, 2.74 g of $FeSO_4·7H_2O$ was dissolved in 1100 mL three-neck flask by adding 830 mL of deionized water (DI). The mixture was stirred for 25 minutes while pure nitrogen was continuously supplied. The CMC solution (1 g/100 mL) was added at a concentration of 0.2%. The CMC-nZVI was made by adding 50 mL of 1.85 g/50 mL of $NaBH_4$ solution dropwise every 5 seconds. After 30 minutes of nitrogen purging, the reaction was resumed, and the resulting suspension was vacuum-filtered and dried at 65 degrees Celsius. The same procedure was repeated for the preparation of Ch-nZVI at the same concentration ratios of nZVI and CMC.

BC-nZVI was synthesized in a chemical reduction; the first steps were followed as outlined before in the preparation of CMC-nZVI. Following 25 minutes of stirring, 0.55 g of biochar was added to the mixture. Then, 50 mL of $NaBH_4$ solution (1.85 g per 50 mL) was prepared to cover the surface of the nZVI particles with biochar. Under nitrogen purging, the reaction was carried out for 30 minutes, and the resulting suspension (BC:nZVI mass ratio of 1:1) was washed with DI water after vacuum filtering, and dried at $70^oC$. The same procedure was repeated for the Synthesis of SC-nZVI at the same concentration ratios of (BC: nZVI mass ratio = 1:1). According to the pathway following a modified protocol of (Ai *et al.* 2007; Üzüm *et al.* 2009).

## 2.4 *Characterization of the synthesized nZVI composites*

The synthesized nZVI composites were characterized for chemical and ultimate analysis. X-ray diffraction (Altima IV, Regaku) was employed to investigate the mineralogy of the materials at 45 kV and 30 mA. Iron nanoparticles were placed in a glass holder and scanned from 20° to 90°. This scan range covered all major species of iron and iron oxides. The scanning rate was set at $2.0°$ min$^{-1}$. The Fourier-transform infrared spectroscopy technique

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(FTIR) was used to determine surface functional groups on the surfaces. Scanning electron microscopy (SEM: FEI, Inspect S50, Netherland) was used to explore the surface morphology. Initially, the samples were coated using nano-gold particles for one min. Thereafter, the samples were examined under an SEM and different images were taken using 30-kV at high vacuum. The Brunauer, Emmett and Teller (BET) method were used to determine the specific surface area and microporosity using nitrogen ($N_2$) at 77 K as the sorbate (ASAP 2020, Micromeritics, USA). The elemental composition of the synthesized nZVI composites was determined by CHNS-O elemental analyzer (series II, PerkinElmer, USA). For the elemental analyses, the contents of hydrogen (H), carbon, (C), sulfur (S), and nitrogen (N) were measured using the instrument, whereas the contents of oxygen (O) were computed using Eq. [1]. The contents of C, H, and O were used to estimate the atomic ratios of O/C and H/C. Laser doppler velocimetery (Zetasizer Nano ZS, Malvern, UK) was employed to measure the average hydrodynamic size for the particles of the different nZVI composites.

$$O\ (\%) = 100-[C\ (\%) + H\ (\%) + N\ (\%) + S\ (\%) + ash\ (\%)] \qquad Eq.\ [1]$$

2.5 *Greenhouse experiments*

 The CMC-nZVI, Ch-nZVI, BC-nZVI, and SC-nZVI composites were added at 0%, 0.5% and 1% (w/w) application rates and combined with 1 kg contaminated soil. Control was added with no amendment. The amended and unamended (control) soils were filled in wet to 60% of the percentage of soil saturation and stored at room temperature ($23 \pm 2$ °C) for 30 days. To guarantee uniformity between the soil and the compounds, it was thoroughly mixed, blending each kilogram of soil separately then agitated. After that, it was moved to the pots to start the greenhouse experiment (Mandal *et al.* 2020). Soil with nZVI composites (CMC-nZVI, Ch-nZVI, BC-nZVI, and SC-nZVI) was evaluated for their ability to immobilize (Cd, Pb, Cu, and Zn) in contaminated soil in a pot greenhouse experiment.  Plastic pots with porous bottom were selected and filled with 1 kg contaminated soil. Amendments (nZVI composites) were added at three different rates including 0%, 0.5%, and 1.0% (w/w). A control treatment was also addedwith no added amendment and soil only. Additionally, the soil was fertilized with 0.0625 g/kg of phosphate ($P_2O_5$), potassium ($K_2O$), and 0.003 g/kg of nitrogen (N) fertilizers (Ren et al.

2021). In each pot 10 seeds of safflower were added, and plant growth was observed regularly to investigate treatment impact on plant growth. Throughout experiment soil moisture in pots was maintained at field capacity and pots were irrigated with tap water to adjust soil moisture level.

2.6 *Plant and soil analyses*

Plants were harvested after a period of 33 days. To harvest plants pots were flushed with excessive water to carefully extract plant roots and shoots. Harvested plants were shifted to lab and analyzed for growth parameters including plant fresh and dry weight and root and shoot length. Latterly plant roots and shoots were analyzed for heavy metals uptake by digesting plants following Ahmad *et al.* 2019)) method. Additionally, after harvesting soil samples were collected from pots and were extracted by AB-DTPA extract to assess available contents of heavy metals.

2.7 *Phytoextraction Indices*

The bioconcentration factor values (BCF) in shoots and roots were computed as shown in Eq. [2] to check phytoextraction and phytostabilization ability (Zhuang *et al.* 2007):

$$(BCF) = \frac{metal\ concentration\ in\ plant\ shoot\ or\ root}{metal\ concentration\ in\ soil} \quad Eq.\,[2]$$

Additionally, the following method was used to determine translocation factor values (TF) as shown in Eq. [3], which represents the capacity of Safflower plants to move HMs from the roots the shoots (McGrath and Zhao 2003; Zacchini *et al.* 2009):

$$(TF) = \frac{metal\ concentration\ in\ aerial\ parts}{metal\ concentration\ in\ root} \quad Eq.\,[3]$$

*Quality control*

Quality control measurements were strictly followed during all experimental activities. High-quality, analytical-grade chemicals were used. In addition, standard procedures for cleaning glassware, testing and calibration of equipment, and measurement accuracy were followed. To ensure quality control and accuracy during the measurement of HMs concentrations, the measurement was repeated three times in 10% of the total number of analyzed soil and leachate samples.

*Statistical analysis*

SPSS program (version 18, SPSS Inc., Chicago, IL) was applied for statistical analysis of the experimental data (SPSS, 2012). The average and standard deviation (±1SD) were applied. The least significant difference (LSD) test at a 5% level of significance was used to evaluate the comparison between averages of treatments.

## 3. Results and discussion

### 3.1 *Characterizations of nZVI composites*

Figure 1 displays the results of the XRD pattern for CMC-nZVI, Ch-nZVI, BC-nZVI, and SC-nZVI. The CMC-nZVI composite's diffraction peaks revealed the presence of nZVI at $2\theta = 44.8°$ and $65.6°$ as well as $Fe_2O_3$ at $2\theta = 30.9°$. In our results, $Fe_2O_3$ diffraction peak was much less intense demonstrating that CMC minimized the oxidation of Fe0 during the preparation process as shown in Figure 1. (Ibrahim *et al.* 2019). Furthermore, the CMC-nZVI composites particles appear to have been small crystallites as can be seen with large diffraction peak (Zhao *et al.* 2019). The XRD pattern of Ch-nZVI revealed considerably nosier baseline diffraction peaks, suggesting amorphous structure (Chen *et al.* 2019). Li *et al.* (2017) reported that diffraction peak at $2\theta = 24.3°$ in the BC-nZVI composites could be due to the presence of carbon. According to Unsoy et al. (2012), the presence of chitosan could be responsible for the sharp peak at $2\theta = 17.2°$, and Fe0 was linked to the diffraction peak at $2\theta = 44.9°$. Furthermore, it was observed that the absence of the diffraction peaks for FeO(OH) and $Fe_2O_3$ in the synthesized Ch-nZVI could be due to the reason that the addition of chitosan reduced the oxidation of $Fe^0$. According to earlier studies (Zou *et al.* 2016; Jin *et al.* 2016), chitosan can function as a stabilizer during the synthesis of a Ch-nZVI composite. The findings show that during the synthesis of the BC-nZVI composite, the oxidation of nZVI nanoparticles was more noticeable on biochar surfaces. These outcomes agree with earlier research by Chen *et al.* (2019). The additional functional groups that contain oxygen (such as carboxyl, hydroxyl, aliphatic, etc.) on the surfaces of BC may be responsible for the increased oxidization of Fe0 during the preparation of BC-nZVI composite. These groups speed up the formation of oxygen-containing complexes

with Fe0, increasing the formation of iron oxide and hydroxide (Lin *et al.* 2010). According to Deon *et al.* (2022), the XRD pattern of the SC-nZVI composite revealed some diffraction peaks that suggested the presence of smectite ($2\theta = 6.2^{\circ}$ and $36.1^{\circ}$), quartz ($2\theta = 26^{\circ}$), and $Fe^0$ ($2\theta = 44.9^{\circ}$). The BC-nZVI composite revealed extra diffraction peaks at $2\theta = 20.3^{\circ}$, $31.1^{\circ}$, and $44.9^{\circ}$, which corresponded to FeO(OH), $Fe_2O_3$, and Fe0, respectively, in contrast to the XRD patterns of the Ch-nZVI. These findings show that during the synthesis of the SC-nZVI composite, nZVI particles were successfully loaded onto the surfaces of the SC.

FTIR spectra in the 400–4000 $cm^{-1}$ wavenumber region was used to examine the surface functional groups of nZVI composites (Figure 2). When biochar and chitosan were successfully loaded onto the surfaces of nZVI during the preparation of BC-nZVI and Ch-nZVI composites, a new characteristic peak at 1360 and 1410 $cm^{-1}$ belonging to the stretching vibration of N–O was observed for the BC-nZVI and Ch-nZVI composites. In comparison to the pristine BC, the stretching vibrations O-H in the BC-nZVI and Ch-nZVI composites were much greater, two peaks were also visible in the FTIR spectra, at 1623 and 2923 $cm^{-1}$, respectively. The generated nZVI composites morphological structure was assessed with (SEM), (Figure. 3) displayed the SEM analyses of the CMC-nZVI, Ch-nZVI, BC-nZVI, and SC-nZVI composites. Ch-nZVI was randomly distributed in the agglomerated formation, while the crystalline on the surface of CMC-nZVI was observed.

The results of FTIR spectra showed distinct bands responsible for various functional groups. O-H vibration stretching is generally responsible for the broad band observed in the FTIR spectra at 3430 cm-1 (Chen *et al.* 2019) corresponding to the aliphatic (C-H) and aromatic rings (C=C) molecules (Lin *et al.* 2020). According to Li *et al.* (2012), the SC-nZVI composite had a peak at 457 cm-1, which is associated with the presence of Si-O. The distinctive peak between 680 and 685 cm-1 could be related to the nZVI and the Fe–O stretching vibration, respectively, which was clearly visible in all the nZVI composites. This indicates that the nZVI had been successfully loaded onto the surfaces of the support materials (CMC, BC, Ch, and SC) (Shu *et al.* 2020; Wei *et al.* 2020). The characteristic peak of C=O was shifted from 1441 cm-1 in pristine BC to 1100 cm-1 in the nZVI composites, which could be due to the attachment of the nZVI particles with the supportive materials (Li *et al.* 2012; Zhu *et al.* 2018). Chen *et al* (2019) suggested that the adsorbed

water and chitosan molecules in both BC-nZVI and Ch-nZVI composites contributed to    292
the increased number of O-H groups.  Because CMC can negatively charge the surfaces of    293
nZVI particles, which greatly increases the electrostatic repulsion between the    294
nanoparticles. On the other hand, nZVI surface modification with CMC led to a decrease    295
in aggregation and a more even dispersion of CMC-nZVI (Karabelli *et al.* 2008). The SEM    296
images demonstrated that BC-nZVI had smaller and more scattered particles, while SC-    297
nZVI surfaces were amorphous, porous, uneven, and rough. Layering of nZVI was    298
observed on the surface of BC, which may offer a rougher surface and more reactive    299
surface area for Cd, Cu, Pb, and Zn sorption. The volatilization of organic components    300
resulted in porous surfaces on the surface structures of the BC-nZVI samples. Deep    301
channels and pores in BC-nZVI started to show more clearly. On the surface of the BC,    302
pores might still be accessible for the adsorption of Cd, Cu, Pb, and Zn (Premarathna *et al.*    303
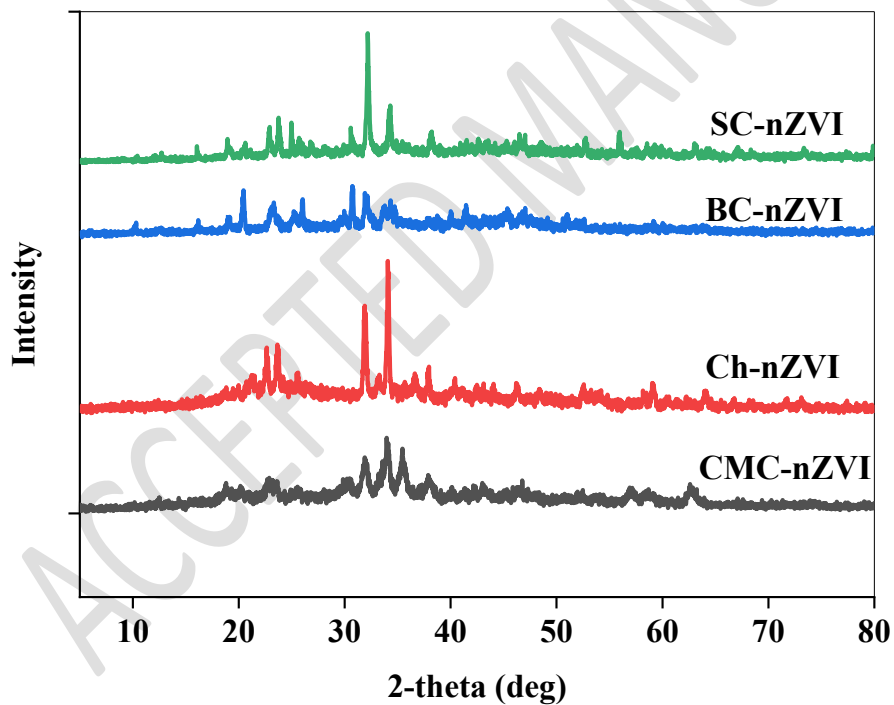2019).    304



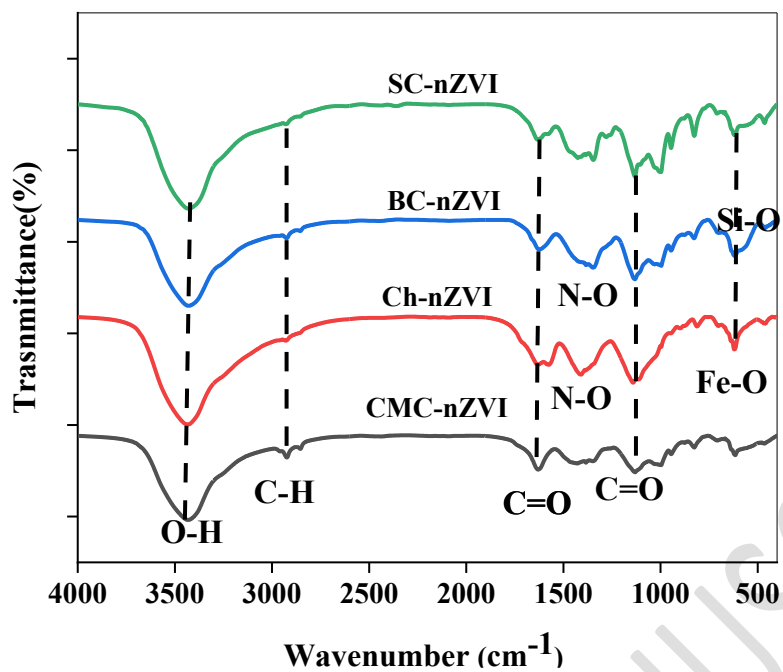**Figure 1**. X-ray diffraction patterns (XRD) of nano-zero valent iron (nZVI) composites    306
with carboxy-methylcellulose (CMC-nZVI), chitosan (Ch-nZVI), biochar (BC-nZVI), and    307
smectite (SC-nZVI).    308

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**Figure 2**. FTIR spectra of nano-zero valent iron (nZVI) composites with carboxy-methylcellulose (CMC-nZVI), chitosan (Ch-nZVI), biochar (BC-nZVI), and smectite (SC-nZVI).
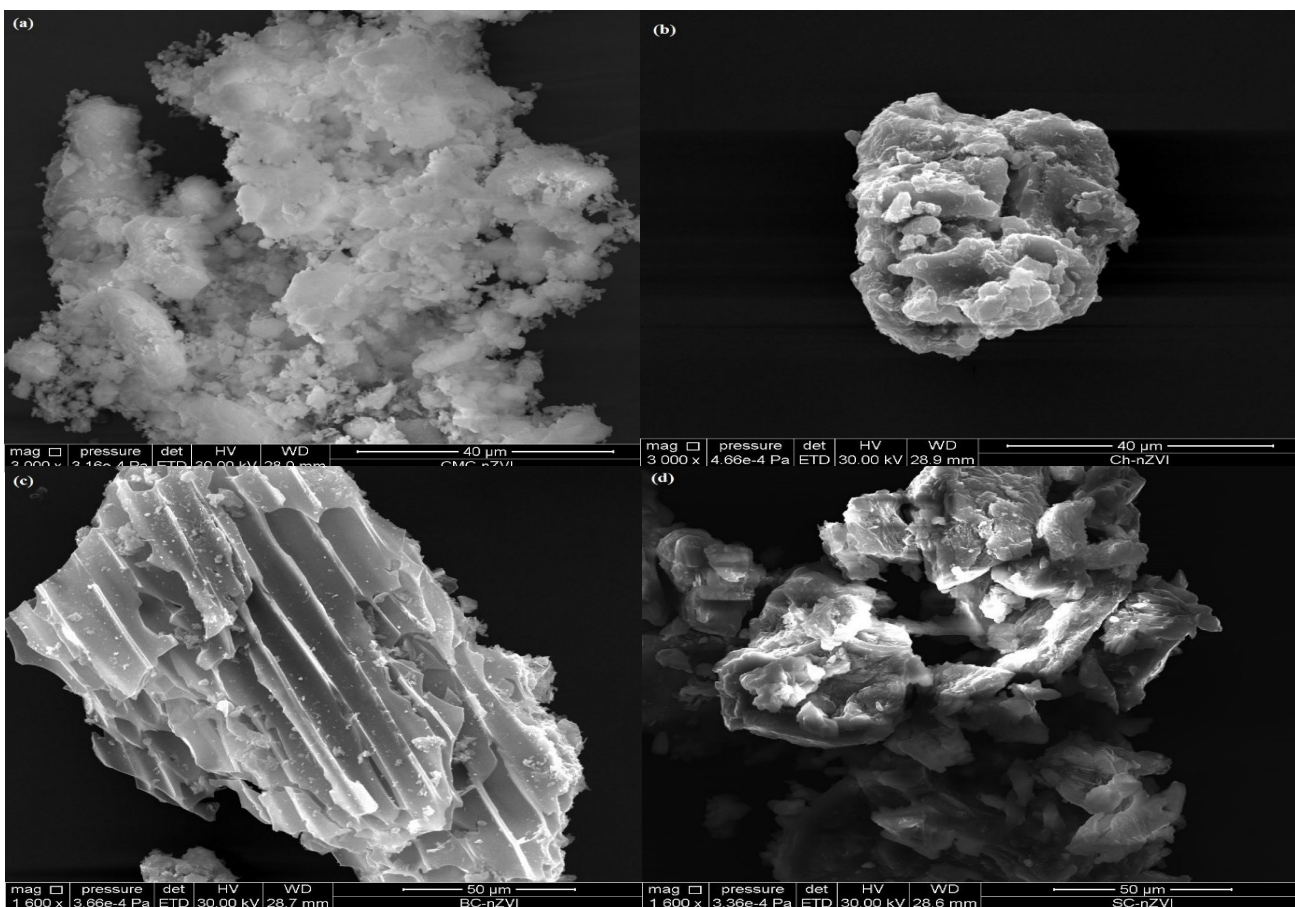
**Figure 3.** Scanning electron microscope images of the nano-zero valent iron (nZVI) 315
composites with (a) carboxy-methylcellulose (CMC-nZVI), (b) chitosan (Ch-nZVI), (c) 316
biochar (BC-nZVI), and (d) smectite (SC-nZVI). 317

Analysis of the elemental composition of the nZVI compounds revealed that the content of 318
carbon (C) decreased significantly from 85.7% in pristine BC to 10.8, 16.3, 14.6, and 1.4% 319
in the CMC-nZVI, Ch-nZVI, BC-nZVI, and SC-nZVI composites, respectively. In 320
contrast, the content of oxygen (O) increased significantly from 7.7% in pristine BC to 321
83.8, 70.6, 76.5, and 96.1% in the CMC-nZVI, Ch-nZVI, BC-nZVI, and SC-nZVI 322
composites, respectively. the higher O contents in the nZVI composites could be attributed 323
to the formation of additional surface functional groups and iron oxide/hydroxide 324
compounds (Wang *et al.* 2017). The content of nitrogen (N) reached 7.7 and 5.1% in the 325
Ch-nZVI and BC-nZVI, respectively, whereas the content of N was almost not detectable 326
in both CMC-nZVI and SC-nZVI composites. The content of sulfur (S) ranged between 1- 327
3% for all composites (Table 2). On the other hand, the pore size of the nZVI decreased 328

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from 68.52 Å to 48.34, 57.85, and 51.50 Å in the CMC-nZVI, Ch-nZVI, and SC-nZVI composites, respectively. These results suggested that the modification of nZVI using the stabilizing (CMC and chitosan) and supporting (BC and SC) materials profoundly altered the surface properties of nZVI, reduced aggregation, and increased the specific surface area of the synthesized nZVI composites (Chen *et al.* 2019; Zhang *et al.* 2019). The BET surface area, pore volume, and pore size of the different nZVI composites were determined using $N_2$ adsorption/desorption isotherms at 77 K (Table 2). For the nZVI composites, the BC-nZVI composite showed a decrease in the specific surface area from 330 to 117 $m^2$ $g^{-1}$, and an increase in pore volume and pore size to 0.1849 cm3 $g^{-1}$ and 62.76 A$^\circ$, respectively.

**Table 2.** Chemical, elemental composition, surface properties, average hydrodynamic size, and zeta potential of the nano-zero valent iron (nZVI) composites with carboxy-methylcellulose (CMC-nZVI), chitosan (Ch-nZVI), biochar (BC-nZVI), and smectite (SC-nZVI)

| Parameter | Unit | CMC-nZVI | Ch-nZVI | BC-nZVI | SC-nZVI |
|---|---|---|---|---|---|
| pH | - | 8.54 | 5.15 | 4.27 | 8.75 |
| C | % | 10.804 | 16.393 | 14.681 | 1.494 |
| H | % | 2.468 | 3.011 | 1.281 | 1.348 |
| N | % | - | 7.699 | 5.124 | - |
| S | % | 2.882 | 2.32 | 2.393 | 1.034 |
| O | % | 83.846 | 70.577 | 76.521 | 96.124 |
| O/C | % | 5.820 | 3.229 | 3.909 | 48.255 |
| H/C | % | 2.741 | 2.204 | 1.047 | 10.827 |
| Surface area | $m^2$ $g^{-1}$ | 19.61 | 13.55 | 117.84 | 36.19 |
| Total volume in pores | $cm^3$ $g^{-1}$ | 0.0237 | 0.0196 | 0.1849 | 0.0466 |
| Pore size | Å | 48.34 | 57.85 | 62.76 | 51.5 |
| Zeta potential | mV | -20.11 | -17.58 | -12.61 | -9.52 |

3.2 *Impacts of stabilized nZVI composites on available heavy metals*

The results demonstrated that the soil additions had a considerable impact on the soil's pH, EC, and soluble Na (Table 3). Compared to control, application of soil additives increased soil pH by 0.12, 0.39, 0.09, 0.31, 0.19, 0.49, 0.31, and 0.43 unit for CMC-nZVI at 0.5%, CMC-nZVI at 1%, Ch-nZVI at 0.5%, Ch-nZVI at 1%, BC-nZVI at 0.5%, BC-nZVI at 1%, SC-nZVI at 0.5%, and SC-nZVI at 1%, respectively. Likewise, the addition of soil additives increased soil EC by 0.36, 0.55, 0.44, 0.40, 0.46, 0.59, 0.61, and 0.67 dS m$^{-1}$ for CMC-nZVI at 0.5%, CMC-nZVI at 1%, Ch-nZVI at 0.5%, Ch-nZVI at 1%, BC-nZVI at 0.5%, BC-nZVI at 1%, SC-nZVI at 0.5%, and SC-nZVI at 1%, respectively, compared to control. The addition of soil additives increased soil soluble Na by 164.54%, 306.38%, 160.99%, 212.77%, 190.07, 331.91%, 140.43%, and 348.94% for CMC-nZVI at 0.5%, CMC-nZVI at 1%, Ch-nZVI at 0.5%, Ch-nZVI at 1%, BC-nZVI at 0.5%, BC-nZVI at 1%, SC-nZVI at 0.5%, and SC-nZVI at 1%, respectively, compared to control. The highest salinity and/or sodium increase was pronounced for SC-nZVI at 1%, followed by BC-nZVI at 1% and CMC-nZVI at 1%.

**Table 3.** Treatment impacts on soil pH, electrical conductivity (EC), and soluble Na

| Treatments | pH | EC dS m$^{-1}$ | Na (mg kg$^{-1}$) |
|---|---|---|---|
| CK | 7.52 de | 0.62 g | 14.1 h |
| CMC-nZVI 0.5% | 7.64 cd | 0.98 f | 37.3 f |
| CMC-nZVI 1% | 7.91 b | 1.17 c | 57.3 c |
| Ch-nZVI 0.5% | 7.61 e | 1.06 d | 36.8 f |
| Ch-nZVI 1% | 7.83 b | 1.02 e | 44.1 d |
| BC-nZVI 0.5% | 7.71 c | 1.08 d | 40.9 e |
| BC-nZVI 1% | 8.01 a | 1.21 b | 60.9 b |
| SC-nZVI 0.5% | 7.83 b | 1.23 b | 33.9 g |
| SC-nZVI 1% | 7.95 b | 1.29 a | 63.3 a |

| | | | |
|---|---|---|---|
| LSD at p =0.05 | 0.12 | 0.03 | 2.12 |

nZVI: nano-zero valent iron; CMC-nZVI: stabilized nZVI composites with carboxy-methylcellulose, Ch-nZVI: stabilized nZVI composites with chitosan, BC-nZVI: stabilized nZVI composites with biochar, and SC-nZVI: stabilized nZVI composites with smectite.

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The results of the available concentration of HMs, which were extracted by AB-DTPA demonstrated that the highest concentration was Zn (408.93–522.20 mg/kg), followed by Cu (95.86–113.85 mg/kg), Pb (48.06–62.66 mg/kg), and Cd (1.23–1.70 mg/kg) However, Cd and Pb concentrations were all below the threshold values (EU/UK/USEPA threshold) for the heavy metal in soil (Adesuyi *et al.* 2015) (Table 3). Our findings demonstrated that, depending on the kind of nZVI composites utilized, the additives substantially impacted the amounts of soil metals. In relation to potential metal extraction from AB-DTPA, the current investigation of soils treated with CMC-nZVI at 1% and Ch-nZVI at 1% showed considerable increases or no significant effect when compared to control.

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**Table 3.** Impacts of stabilized nano-zero valent iron (nZVI) composites with carboxy-methylcellulose (CMC-nZVI), chitosan (Ch-nZVI), biochar (BC-nZVI) biochar, and smectite (SC-nZVI) on the availability of heavy metals in soil (mg/kg).

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| Treatments | AB-DTPA extractable HMs | | | |
|---|---|---|---|---|
| | Cd | Cu | Pb | Zn |
| CK | 1.23 b | 95.85 b | 49.90 de | 431.27 cd |
| CMC-nZVI 0.5% | 1.48 ab | 102.81 ab | 56.77 abc | 477.73 abc |
| CMC-nZVI 1% | 1.70 a | 113.17 a | 62.66 a | 522.20 a |
| Ch-nZVI 0.5% | 1.38 b | 104.94 ab | 58.80 ab | 476.47 abc |
| Ch-nZVI 1% | 1.37 b | 113.15 a | 60.41 ab | 501.73 ab |
| BC-nZVI 0.5% | 1.25 b | 100.73 ab | 50.98 cde | 420.66 cd |

| | | | |
|---|---|---|---|
| BC-nZVI 1% | 1.24 b | 94.74 b | 48.06 e | 408.93 d |
| SC-nZVI 0.5% | 1.41 ab | 102.87 ab | 55.45 bcd | 460.37 bcd |
| SC-nZVI 1% | 1.38 b | 105.34 ab | 54.52 bcde | 448.60 bcd |
| LSD at p = 0.05 | 0.29 | 14.82 | 6.86 | 58.48 |

CK: control; CMC-nZVI at 0.5% and 1%: Carboxy-methylcellulose with nZVI at dosage at 0.5 and 1 % respectively; Ch-nZVI at 0.5% and 1%: Chitosan with nZVI at dosage at 0.5 and 1 % respectively; BC-nZVI at 0.5% and 1%: biochar with nZVI at dosage at 0.5 and 1 % respectively; SC-nZVI at 0.5% and 1%: smectite with nZVI at dosage at 0.5 and 1 % respectively; LSD: Different letters indicate significant differences.

### 3.3 *Impacts of stabilized nZVI composites on plant growth*

Figure 4 shows the impact of a treatment on the fresh weights and dry weights of the roots and shoots of a safflower plant's growth in a pot experiment. The results showed that SC-nZVI at 1% and BC-nZVI at 1% inhibited seed germination, but no plant grew in both treatments. Additionally, most other treatments significantly de-creased the biomass weight of plant shoots and plant length. For example, com-pared to the control, the addition of CMC-nZVI 0.5%, CMC-nZVI 1%, Ch-nZVI 0.5%, Ch-nZVI 1%, BC-nZVI 0.5%, BC-nZVI 1%, SC-nZVI 0.5%, and SC-nZVI 1% resulted in approximately 51.5%, 66.7%, 89%, 65.7%, 59%, 100%, 15.2%, and 100% decreases in the fresh weight of shoots, respectively. Meanwhile, com-pared to the control, the addition of CMC-nZVI 0.5%, CMC-nZVI 1%, Ch-nZVI 0.5%, Ch-nZVI 1%, BC-nZVI 0.5%, BC-nZVI 1%, SC-nZVI 0.5%, and SC-nZVI 1% resulted in approximately 39.6%, 38.6%, 11.9%, 35.5%, 30.4%, 100%, 11.4%, and 100% decreases in the dry weight of shoots, respectively. Research in this field has shown that nZVI has distinct biological impacts on the growth and development of plants, emphasizing the fact that nZVI has strong dosage inhibition at low doses and negative excitatory effects at higher doses (Cui *et al.* 2023; Baragano *et al.* 2022; Yang *et al.* 2022). Yang *et al.* (2023), found that (nZVI) generates negative effects on the growth of maize, acting as hazardous. According to our findings, the additions raised the

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concentrations of soluble sodi-um and soil salinity, which may have contributed to the 393
detrimental impacts of the additives containing nanoscale zero-valent iron on plant growth. 394
The correlation study showed that there is a significant negative correlation between plant 395
growth parameters (biomass weight or plant length) and soil properties (pH, EC, or soluble 396
Na). A mediated decrease in nitrogen uptake may be the cause of the decrease in plant dry 397
matter (Pradas-del-Real *et al.* 2013), or the distortion of the structure of plant roots, which 398
subsequently strained the plants' ability to absorb nutrients and grow vigorously (Ali *et al.* 399
2013; Gill *et al.* 2014). The nature of the materials and the type of soil employed in this 400
study most likely contributed to the distortion of the plant roots' structure (Rajkovich *et al.* 401
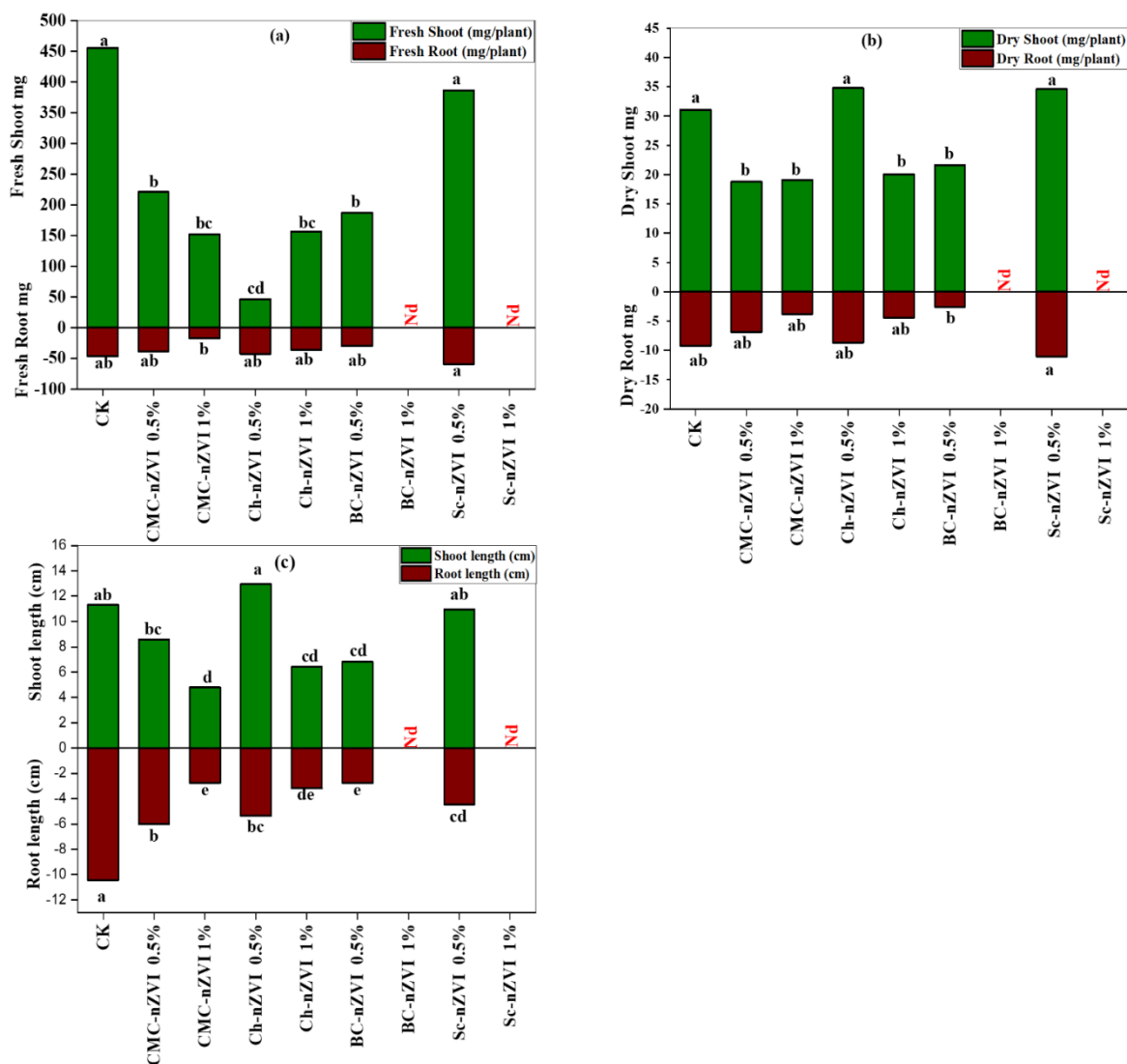2012). 402

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**Figure 4. (a)** Fresh weight of shoots and roots of plants; (b) dry weight of shoots and roots of plants; (c) length of plant shoots and roots as affected by stabilized nano-zero valent (nZVI) composites with carboxy-methylcellulose (CMC-nZVI 0.5%, CMC-nZVI 1%), chitosan (Ch-nZVI 0.5%, Ch-nZVI 1%), biochar (BC-nZVI 0.5%, BC-nZVI 1%), smectite (SC-nZVI 0.5%, and SC-nZVI 1%) respectively.

3.4 *Treatment impacts of HMs concentration and uptake in plants*

 Table 4 shows the HMs concentrations in plant shoots and roots. The results showed that shoot Cd and Pb, as well as root Cd concentrations, were under the detection limit of ICP. The Cu and Zn concentrations in shoots significantly increased by 182 and 114% in plants treated with CMC-nZVI, respectively, by 1% compared to the control. On the contrary,

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compared to the control, the Cu concentrations in roots significantly decreased by 65.2% in plants treated with Ch-nZVI 1%. Similarly, adding Ch-nZVI 1% and BC-nZVI 0.5% significantly reduced root Zn concentrations by 31.4 and 27.4%, respectively. Adding CMC-nZVI 0.5% and BC-nZVI 0.5% significantly decreased Pb concentrations by 47.9 and 85.5% compared to control, respectively. Additionally, both CMC-nZVI 1% and Ch-nZVI 1% decreased root Pb concentration from 89.46 mg/kg to under the detection limit (UDL) of ICP. It was observed that the concentrations of HMs in shoots were below the permissible limit, suggested lower translocation of HMs from roots to shoots (Gupta *et al.* 2006).

**Table 4.** Concentrations of heavy metals in plant shoots and roots as affected by stabilized nano-zero valent iron (nZVI) composites with carboxy-methylcellulose (CMC-nZVI), chitosan (Ch-nZVI), biochar (BC-nZVI) biochar, and smectite (SC-nZVI).

| | Shoot concentrations (mg/kg) | | | | Root concentrations (mg/kg) | | | |
|---|---|---|---|---|---|---|---|---|
| | Cd | Cu | Pb | Zn | Cd | Cu | Pb | Zn |
| CK | UDL | 27.24 b | UDL | 239.86 b | UDL | 485.94 a | 89.46 a | 2647.64 a |
| CMC-nZVI 0.5% | UDL | 35.63 ab | UDL | 283.42 b | UDL | 479.29 a | 46.60b | 2647.49 a |
| CMC-nZVI 1% | UDL | 76.94 a | UDL | 512.35 a | UDL | 355.89 ab | UDL | 2525.38 ab |
| Ch-nZVI 0.5% | UDL | 43.07 ab | UDL | 383.61 ab | UDL | 482.50 a | 113.54 a | 2722.25 a |
| Ch-nZVI 1% | UDL | 30.79 b | UDL | 342.37 ab | UDL | 168.99 b | UDL | 1816.37 c |
| BC-nZVI 0.5% | UDL | 47.81 ab | UDL | 367.94 ab | UDL | 331.61ab | 12.95 bc | 1920.98 bc |
| BC-nZVI 1% | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd |
| SC-nZVI 0.5% | UDL | 17.82 b | UDL | 286.94 b | UDL | 355.44 ab | 118.48 a | 2324.26 abc |
| SC-nZVI 1% | Nd | Nd | Nd | Nd | Nd | Nd | Nd | Nd |
| LSD at p = 0.05 | – | 44.90 | – | 216.83 | – | 206.43 | 39.14 | 638.17 |

CK: control; CMC-nZVI 0.5% and 1%: Carboxy-methylcellulose with nZVI at dosage 0.5 and 1 % respectively; Ch-nZVI 0.5% and 1%: Chitosan with nZVI at dosage 0.5 and 1 % respectively; BC-nZVI 0.5% and 1%: biochar with nZVI at dosage 0.5 and 1 % respectively; SC-nZVI 0.5% and 1%: smectite with nZVI at dosage 0.5 and 1 % respectively; UDL: under detection limit; Nd: no plants; LSD: Different letters indicate significant differences.

Table 5 shows that HMs uptake to shoot and root significantly (p < 0.05) increased with
the Ch-nZVI at 0.5% and SC-nZVI at 0.5% application compared to the control.
Application rate of 0.5% for Ch-nZVI and SC-nZVI increased the shoot uptake of Cu by
81.3% and 17.3%, and Zn by 80.9% and 34.1%, respectively, compared to the control,
while showing no significant differences compared to the control. The levels of Cd and Pb
in shoots were generally low, ranging from zero mg/kg in many plants to UDL in others,
and well below the EU, UK, and USEPA thresholds for heavy metals in plants, the
parameters were provided by the (EU/UK/USEPA threshold) (Adesuyi *et al*. 2015).

**Table. 5.** Uptake of heavy metals by the plant shoots and roots as affected by stabilized
nano-zero valent iron (nZVI) composites with carboxy-methylcellulose (CMC-nZVI),
chitosan (Ch-nZVI), biochar (BC-nZVI) biochar, and smectite (SC-nZVI).

| Treatment | uptake of Cd (ug/plant$^{-1}$) | uptake of Cu (ug/plant$^{-1}$) | uptake of Pb (ug/plant$^{-1}$) | uptake of Zn (ug/plant$^{-1}$) |
|---|---|---|---|---|
| uptake by shoots | | | | |
| CK | UDL | 0.8567 ab | UDL | 7.4833 b |
| CMC-nZVI 0.5% | UDL | 0.6483 ab | UDL | 5.1342 bc |
| CMC-nZVI 1% | UDL | 1.4117 a | UDL | 9.5131 ab |
| Ch-nZVI 0.5% | UDL | 1.5542 a | UDL | 13.5375 a |
| Ch-nZVI 1% | UDL | 0.6139 ab | UDL | 6.8264 b |
| BC-nZVI 0.5% | UDL | 0.7911 ab | UDL | 8.0256 ab |
| BC-nZVI 1% | Nd | Nd | Nd | Nd |
| SC-nZVI 0.5% | UDL | 0.9572 b | UDL | 10.0389 ab |
| SC-nZVI 1% | Nd | Nd | Nd | Nd |
| uptake by Roots | | | | |
| CK | UDL | 4.5567 ab | 0.9067 a | 24.6267 ab |
| CMC-nZVI 0.5% | UDL | 3.3050 abc | 0.3542 a | 18.4233 abc |
| CMC-nZVI 1% | UDL | 1.3764 abc | UDL | 9.6078 abc |
| Ch-nZVI 0.5% | UDL | 4.3889 bc | 0.8167 a | 25.1069 ab |
| Ch-nZVI 1% | UDL | 0.8097 bc | UDL | 8.1403 bc |
| BC-nZVI 0.5% | UDL | 0.8533 bc | 0.0333 a | 4.9433 c |
| BC-nZVI 1% | Nd | Nd | Nd | Nd |
| SC-nZVI 0.5% | UDL | 5.0917 a | 0.8333 a | 28.3000 c |
| SC-nZVI 1% | Nd | Nd | Nd | Nd |

CK: control; CMC-nZVI 0.5% and 1%: Carboxy-methylcellulose with nZVI at dosage of 0.5
and 1 % respectively; Ch-nZVI at 0.5% and 1%: Chitosan with nZVI at dosage of 0.5 and 1 %
respectively; BC-nZVI at 0.5% and 1%: biochar with nZVI at dosage of 0.5 and 1 %

respectively; SC-nZVI at 0.5% and 1%: smectite with nZVI at dosage of 0.5 and 1 % respectively; UDL: under detection limit; Nd: no plant.

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Table 6 displays the phytoextraction indices that were computed for this investigation. The TF is calculated as the ratio metal concentration in the shoots and the roots. It is used to indicate the transfer of metal from the roots to the shoots. While the BCF value indicates the metal concentration in the shoot to that in the soil. Our results exhibited that TF was <1 in all treatments, suggesting that the absorbed concentrations of Cu, Pb, and Zn accumulated in plants roots, and did not translocate to the shoots. Our finding demonstrated that there was no transfer of Pb from roots to shoots. The higher TF values in all amended soil showed a greater translocation of Cu and Zn from plant roots toward plant shoots. In comparison with the control, CMC-nZVI 1% and Ch-nZVI 1% showed a higher translocation of Cu and Zn from roots to shoots, respectively. On the other hand, a 100% reduction in TF values was observed for Cd and Pb after application of all the amendments. According to previous study by Usman *et al.* (2013) and Lu *et al.* (2014), phytoextraction indices for plant species such as TF and BCF with values larger than unity are typically considered to be hyperaccumulators, while TF and BCF values less than unity are typically considered to be excluders. Plants with TF > 1 are classified as hyperaccumulators, and those with TF < 1 are called excluders (Usman and Mohammed 2009). Overall, application of the amendments increased the phytoremediation indices; however, the increments were below unity, suggesting unsuitability as hyperaccumulators for practical application.

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**Table 6.** Treatment effects on the shoot-to-root ratio, bioconcentration factor values (BCF) and the adsorbed metal by the adsorbed shoots/metal of the entire plant (%).

| Treatment | CK | CMC-nZVI 0.5% | CMC-nZVI 1% | Ch-nZVI 0.5% | Ch-nZVI 1% | BC-nZVI 0.5% | BC-nZVI 1% | SC-nZVI 0.5% | SC-nZVI 1% |
|---|---|---|---|---|---|---|---|---|---|
| **Shoot-to-root ratio (TF)** | | | | | | | | | |
| Cd | 0 | 0 | 0 | 0 | 0 | 0 | Nd | 0 | Nd |
| Cu | 0.056 bc | 0.074 bc | 0.212 ab | 0.085 bc | 0.397 a | 0.053 bc | Nd | 0.063 bc | Nd |
| Pb | 0 | 0 | 0 | 0 | 0 | 0 | Nd | 0 | Nd |
| Zn | 0.091 bc | 0.106 ab | 0.199 a | 0.139 ab | 0.195 a | 0.066 bc | Nd | 0.130 ab | Nd |
| **Bioconcentration factor values Shoot (BCF)** | | | | | | | | | |
| Cd | 0 | 0 | 0 | 0 | 0 | 0 | Nd | 0 | Nd |
| Cu | 0.035 bc | 0.045 abc | 0.098 a | 0.055 ab | 0.039 bc | 0.041 bc | Nd | 0.023 bc | Nd |
| Pb | 0 | 0 | 0 | 0 | 0 | 0 | Nd | 0 | Nd |
| Zn | 0.072 b | 0.085 b | 0.154 a | 0.115 ab | 0.103 ab | 0.111 ab | Nd | 0.086 b | Nd |
| **Bioconcentration factor values Root (BCF)** | | | | | | | | | |
| Cd | 0 | 0 | 0 | 0 | 0 | 0 | Nd | 0 | Nd |
| Cu | 0.620 a | 0.612 a | 0.454 ab | 0.616 a | 0.216 bc | 0.141 c | Nd | 0.454 ab | Nd |
| Pb | 0.796 ab | 0.796 ab | 0.760 ab | 0.819 a | 0.546 b | 0.193 c | Nd | 0.699 ab | Nd |
| Zn | 0.271 a | 0.141 ab | 0.000 | 0.229 a | 0.000 | 0.013 b | Nd | 0.120 ab | Nd |

nZVI: nano-zero valent iron; CMC-nZVI: stabilized nZVI composites with carboxy-methylcellulose, Ch-nZVI: stabilized nZVI composites with chitosan, BC-nZVI: stabilized nZVI composites with biochar, and SC-nZVI: stabilized nZVI composites with smectite.

# 4. Conclusion

In this work, nanoscale zero-valent iron (nZVI) through synthesis with CMC (CMC-nZVI), chitosan (Ch-nZVI), biochar (BC-nZVI), and smectite (SC-nZVI) were applied in polluted soils cultivated with safflower were evaluated in minimizing the risks of heavy metals in mining soils. The results showed that nZVI compounds significantly increased soil pH, EC, and soluble Na. Also, the applied materials had no significant effect on reducing the contents of HMs in the aboveground biomass of safflower plants. However, the application of Ch-nZVI, BC-nZVI, or SC-nZVI had no significant effect and led to a decrease in the concentration of heavy metals in the rhizosphere, especially for Pb, Cu, and Zn as well as

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a narrow but non-significant increase in plant weight. The applied modifications were not feasible for the immobilization of HMs or extraction by safflower plants. Due to the negative effects of composite materials, it can be concluded that the application of nZVI composites to soil may be limited and can only be used in aquatic environments.

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## Author Contributions

MAM and ARU conceptualized the project, developed methodology, and written the draft, ASA supervised the project, MIA acquired funding and managed resources, HMI validated the results, ARU collected soil samples, HAA conducted the analyses, MA helped in lab investigation, MIR help conduct the analyses, whereas ASA, MIA, HAA, MA, and MIR reviewed and edited the manuscript.

**Conflicts of Interest**: The authors declare that there is no conflict of interest.

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