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ORIGINAL PAPER

Nano- and microscale iron for Fe fortification in *Spinacia oleracea*

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Abstract
The effectiveness of nanoscale zero-valent iron (NZVI) and microscale zero-valent iron (MZVI) in fortifying spinach (*Spinacia oleracea*) with iron was examined. The changes in uptake of some macro- and microelements essential for plants and humans were also investigated in the presence of NZVI and MZVI. Spinach was grown hydroponically until maturity using three doses of iron (11, 55, 110 mg/L) using NZVI, MZVI, and ferrous sulfate ($FeSO_4$). Spinach produced most biomass when exposed to 55 mg/L NZVI and 110 mg/L MZVI. With the application of 55 mg/L NZVI, the biomass increase in the edible part (the aboveground biomass) was ~ 1.10-fold compared to 110 mg/L MZVI, and ~ 1.57-fold compared to 55 mg/L $FeSO_4$ treatments. There was a 1.15-fold increase in iron content in spinach treated with 55 mg/L NZVI compared to 110 mg/L MZVI and a 1.70-fold increase was seen with 55 mg/L NZVI compared to 55 mg/L $FeSO_4$. NZVI and MZVI also enhanced the plant uptake of some macronutrients (P, K, S, Ca, Mg, Na) and micronutrients (Zn, Mn, Cu, B). Both NZVI and MZVI have the potential for use as nutrient fortifiers in crops.

Keywords Nanoscale zero-valent iron · Microscale zero-valent iron · Biofortification · Spinach biomass · Iron uptake

Introduction

Iron (Fe) is one of the key micronutrients for plant, human, and animal nutrition. It plays a major role in the catalysis of enzymatic reactions in the cells [1, 2]. Even though it is one of the most abundant elements on earth (35% of its mass and 5.2% of its crust), ~ 30% of the world's soils are deficient in plant-available iron [3]. Among the micronutrients, plants need iron the most as it is a major constituent of several enzymes and pigments [4]. Iron takes part in the reduction of nitrate and sulfate and the production of energy within the plant [4]. Iron is also essential for chlorophyll formation [4]. Humans and animals are dependent on plants for their iron requirements. Iron is vital for oxygen transport in the body as well as for energy metabolism [5]. Iron constitutes the functional core of the heme complex in hemoglobin (oxygen carrier in the blood) and myoglobin (oxygen storage unit in muscles) [5]. It is also found in the catalytic center of cytochromes which perform redox reactions [4].

Iron deficiency is one of the most common human nutritional deficiencies prevalent across the globe and a leading factor for disabilities and deaths [6]. Iron deficiency leads to anemia in humans [7]. Anemia is a condition in which our blood lacks enough red blood cells (RBCs) and, thus, fails to carry adequate oxygen to the body tissues. While there are other causes of anemia, iron deficiency in the body is the most common one [8]. Bone marrow needs iron to make hemoglobin which in turn leads to a human having healthy RBCs. It was estimated (2019) that around 39.8% (269 million) of children (aged < 5 years), 36.5% (32 million) of pregnant women, and 29.6% (539 million) of non-pregnant women in the world suffer from anemia [9]. Children in the African Region form the highest proportion (> 60%) of individuals suffering from anemia while the most affected number of women reside in the Southeast Asia Region where 244 million women of reproductive age (15–49 years) are affected [9]. It is imperative to develop nutrient security strategies that will help humans to overcome iron deficiency.

Several interventions are practiced to combat iron deficiency in humans. The most effective intervention to

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alleviate iron deficiency is dietary diversification which includes consumption of meat, vegetable, fish, and fruits with staple food [10]. Interventions with micronutrients in tablet and sachet forms, and food fortification through the addition of minerals to processed food are also practiced [2, 10]. However, biofortification is proposed as a more efficient and cost-effective way to increase iron content in food crops and, thus, make an impact on human health. Biofortification involves making a high amount of nutrient elements naturally available in the edible parts of crops [11]. It is achieved by fertilization, conventional breeding, and/or genetic engineering [2]. It is also done with microorganisms (for example, biofortification of selenium (Se) in wheat) [12]. The fortification of staple crops with bioavailable iron is likely to provide a sustainable solution to Fe deficiency in humans [13–15].

Agronomic interventions, however, are not always efficient because the iron in oxidized ferric form is less soluble in aerobic environments. The oxidized iron, Fe(III), has a very low solubility at basic pH and in the presence of high bicarbonate (HCO_3^-) and that leads to reduced uptake of iron by plant roots [16]. Even though iron is the fourth abundant element in the earth's crust, it is the third most limiting nutrient for plant growth [10]. Over the last two decades, innovative interventions involving soil and foliar application of iron-containing compounds have been tried on maize, wheat, barley, soybean, common beans, oats, and leafy vegetables (e.g., spinach) to increase the iron content in the edible parts[15, 17–23].

Of late, there has been an increased interest in the application of nanomaterials for agronomic purposes. Nanomaterials have been used as smart delivery systems of fertilizers, herbicides, pesticides, and plant growth regulators [24]. Scientists have also explored the possibility of using nanoparticles to biofortify crops. Nanoparticles are applied to enhance the growth of plants which in turn leads to the increased uptake of mineral elements. Nanoscale zero-valent iron (NZVI) was used for phosphate removal and the phosphate-containing spent NZVI was subsequently used as a source of phosphorus and iron for algae and spinach growth [17]. Increased growths of algae and spinach were observed when phosphate-sorbed NZVI was used as the sole source of iron and phosphorus [17]. The iron content increased significantly in all plant parts (roots, stems, and leaves) in that study [17]. In another study, the iron content was found to increase in spinach in a dose-dependent manner where spinach was hydroponically grown using iron oxide nanoparticles [25]. Iron oxide nanoparticles were also found to significantly enhance the chlorophyll contents in subapical leaves in hydroponically grown soybeans, and toxicity was not observed [26]. Black-eyed peas had 34% more iron content in leaves compared to the control when the foliar application of iron nanoparticles was tried [27]. Iron nanoparticles at a very low concentration (1.3 mg/L) were found to promote the growth of capsicum [1], and they were found to modify the leaf organization, chloroplast number, and grana stacking as well as govern the development of vascular bundles [1].

This study examined whether nanoscale zero-valent iron (NZVI) can be used for iron biofortification in hydroponically grown spinach (*Spinacia Oleracea*) and whether the presence of NZVI affects the plant uptake of other minerals. Microscale zero-valent iron (MZVI) particles were used in one of the treatments for a meaningful comparison. Spinach was selected for this study as it is one of the most consumed leafy vegetables with a global cultivation area of 930,791 ha and production of 30,107,231 tons [28]. Further, spinach is fast growing, and readily available worldwide. It is a source of iron, calcium, and many vitamins (mainly vitamin A), making it an important crop for human nutrition [29]. We hypothesized that NZVI would effectively fortify spinach with iron compared to other available fortifiers (MZVI and iron sulfate) without adversely affecting the plant uptake of other essential minerals.

Experimental section

Chemicals

Iron(II) sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$, 99% pure, Alfa Aesar), microscale zero-valent iron powder (< 10 microns, 99.9 + %, Sigma-Aldrich), sodium borohydride ($NaBH_4$, 98%, Sigma-Aldrich), sodium hydroxide (5 N NaOH, Alfa Aesar), HNO_3 (68%, J.T. Baker), methanol (production grade, BDH), ethanol (ACS grade, Mallinckrodt Chemicals), calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, ACS grade, Alfa Aesar), potassium nitrate (KNO_3, AR grade, Mallinckrodt Chemicals), magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$, ACS grade, Mallinckrodt Chemicals), magnesium nitrate hexahydrate ($Mg(NO_3)_2{\cdot}6H_2O$, ACS grade, Alfa Aesar), ammonium dihydrogen phosphate ($NH_4H_2PO_4$, ACS grade, Alfa Aesar), sodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$, ACS grade, Ameresco), copper(II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$, ACS grade, BDH), manganese sulfate monohydrate ($MnSO_4{\cdot}H_2O$, ACS grade, Mallinckrodt Chemicals), sodium molybdate dihydrate ($Na_2MoO_4{\cdot}2H_2O$, ACS grade, BTC), and zinc sulfate monohydrate ($ZnSO_4{\cdot}H_2O$, ACS grade, J.T. Baker) were used as received unless otherwise specified.

Synthesis and preparation of NZVI

NZVI Synthesis. NZVI particles were synthesized using the sodium borohydride reduction method [30]. Iron(II) sulfate heptahydrate (10 g) was dissolved in 100 mL of 30% of methanol (30 mL methanol + 70 mL deoxygenated deionized

(DI) water) (Solution A). The pH of the solution was then adjusted to 6.1 by dropwise addition of 5 N NaOH. In the meantime, 3.94 g of sodium borohydride was dissolved in 100 mL of deoxygenated DI water in a 100 mL volumetric flask (Solution B). Once the pH reached 6.1, Solution A was added dropwise using a burette to Solution B under vigorous stirring conditions (using a magnetic stirrer). The combined solution was then allowed to stand for 20 min. The resultant black precipitates (NZVI) were centrifuged and washed with ethanol. The NZVI in slurry form was then dried in a vacuum oven for 24 h under N_2 environment. Finally, the dried NZVI clusters were ground into powder form using a mortar and pestle and stored in 20-mL amber glass vials (headspace flushed with nitrogen) for later use.

Experimental setup for spinach study in hydroponics

Germination and plant preparation for the hydroponic experiment

Spinach (Double Choice Hybrid, *Spinacia oleracea*, Burpee, Warminster, PA) seeds were purchased from a local store. Seeds were initially treated with liquid nitrogen for cracking up the shells for faster germination. The spinach seeds were then placed on Perlite media in Petri dishes and kept in the dark at room temperature (22 ± 2 °C) for three days for germination. After three days, the Petri dishes were moved to a custom-made plant growth chamber (Fig. 1a) and germinated seedlings were allowed to grow for additional three days. The seedlings were provided with 100 µmol/m^2/s intensity cool-white fluorescent light (14 h light/10 h dark cycle).

Growth studies

After 3 d of growth in the growth chamber, the spinach seedlings were ready for transplantation to the hydroponic growth reactors. The growth reactors were 18.4 cm tall plastic tumblers with a top diameter of 8.9 cm (Fig. 1b). The roots of spinach plants seedlings were washed with copious amounts of deionized (DI) water before transplantation. Three spinach seedlings were then placed into a Styrofoam disk float (three holes in the disk) with their roots below the disk and the shoots supported above with a wrap of non-absorbent cotton [31].

Hoagland nutrient solution [32] was modified according to the treatments of the experiment. For the NZVI and MZVI experiments, a measured amount of the specific iron particles was added as the only iron source in the Hoagland solution. For the $FeSO_4$ experiment, a measured amount of salt was added as the only iron source in the Hoagland solution. The nutrient solution which was used as the negative control (Control) contained no iron (Table 1).

The experiment was completely randomized with three treatments (11, 55, and 110 mg Fe/L), each with nanoparticles (NZVI), microparticles (MZVI), and ferrous sulfate ($FeSO_4$) treatments. A control was run with no iron addition. Each treatment and the control had three replicates (a total of 30 reactors). A measured amount (450 mL) of the nutrient solution (Table 1) was transferred to each reactor (tumbler), and iron was added to each reactor (no iron addition in the control units). The Styrofoam disk (with the spinach seedlings) was now placed in the reactor, ensuring that it floated on water and there was continuous root contact with the nutrient solution. The growth reactors were wrapped with aluminum foil to prevent light in the root zone and, thus, simulate actual growth conditions. The solution was aerated with compressed air (~2 cm^3/min)

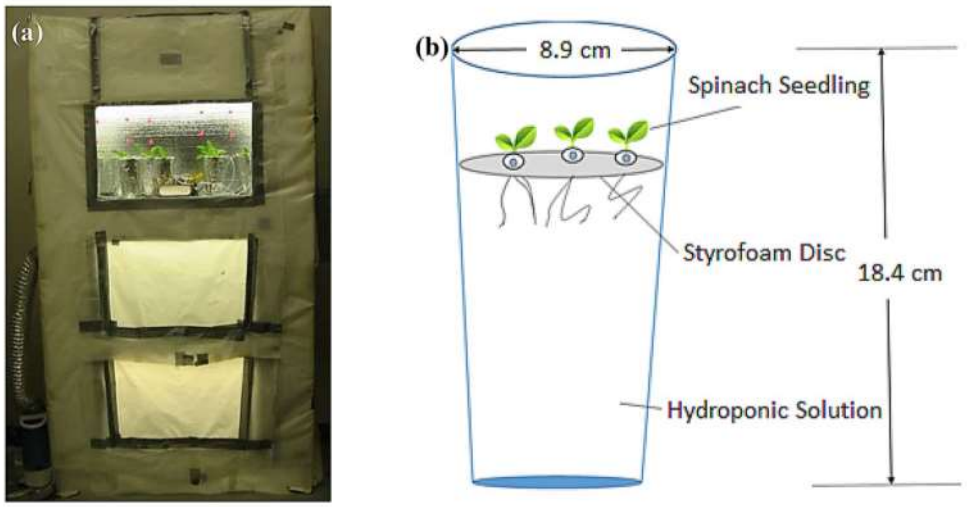

Fig. 1 **(a)** Custom-made growth chamber used for the spinach study. The temperature was ~ 75 °F and relative humidity was maintained ~ 60%; **(b)** schematic of a growth reactor used in this study

Table 1 Composition of modified Hoagland solution containing no iron

Chemicals	Final concentration		Important ions
	mM or μM	mg/L	
$Ca(NO_3)_2{\cdot}4H_2O$	2 mM	472	Ca^{2+}, NO_3^-
KNO_3	6 mM	606	K^+, NO_3^-
$MgSO_4{\cdot}7H_2O$	0.5 mM	123	Mg^{2+}, SO_4^{2-}
$Mg\ (NO_3)_2{\cdot}6H_2O$	0.5 mM	128	Mg^{2+}, NO_3^-
$NH_4H_2PO_4$	2 mM	230	NH_4^+, $H_2PO_4^-$
$Na_2B_4O_7{\cdot}10H_2O$	20 μM	3.81	$B_4O_7^{2-}$
$CuSO_4{\cdot}H_2O$	0.5 μM	0.089	Cu^{2+}
$MnSO_4{\cdot}3H_2O$	10 μM	2.05	Mn^{2+}
$Na_2MoO_4{\cdot}2H_2O$	0.5 μM	0.12	MoO_4^{2-}
$ZnSO_4{\cdot}H_2O$	4 μM	0.716	Zn^{2+}

to provide oxygen to the roots and also to keep the iron particles in suspension [33]. The growth solution in each reactor was replaced every five days and fresh iron was added. The plants were grown in the growth chamber fitted with cool-white fluorescent plant-grow bulbs (intensity of 100 μmol/m^2/s) in a 14-h light/10-h dark cycle.

Spinach study

Plants were harvested after 39 days of hydroponic growth. The harvested plants were washed with copious amounts of DI water and the roots were again washed with 10 mM $CaCl_2$ solution to remove any iron particles attached to the root surface [17]. The three plants grown in each reactor were kept together and marked for later identification. Then, the plants from each reactor were separated into two parts—the roots (R) and the aboveground part (AP) (Fig. 2). The AP consisted of everything (stem and leaves) excluding the roots. The separated plant materials were then dried at 65 °C until constant weight. After drying, the R and AP biomass were weighed. The roots (R) yielded very little biomass and were not analyzed for macro- and microelements. Discarding the root did not affect this study as spinach root is not typically consumed by humans. The AP samples were then powdered and homogenized using a mortar and pestle using liquid nitrogen.

Macro- and micronutrient measurements

The powdered plant samples were digested using a standard protocol [34]. Briefly, the samples (0.25 g) were weighed into a digestion tube and 5.0 mL of 16 M HNO_3 was added. The mouth of the digestion tube was covered with a watch glass, and the tube was allowed to stand overnight. The tube was then placed on a block digester and digested at 125 °C for 1 h, cooled to room temperature, and 3 mL of 30% H_2O_2 was added to the tube. The content was again digested at 125 °C until the digest was clear. The colorless digest was brought to volume in a 50-mL volumetric flask by adding 1:10 HNO_3. The solution was then analyzed for P, K, Ca, Mg, Na, Zn, Fe, Mn, Cu, and B using a Perkin Elmer ICP-OES (5300 DV Model). A control standard was run after every ten samples to check whether the values were within acceptable limits (10% of the expected values).

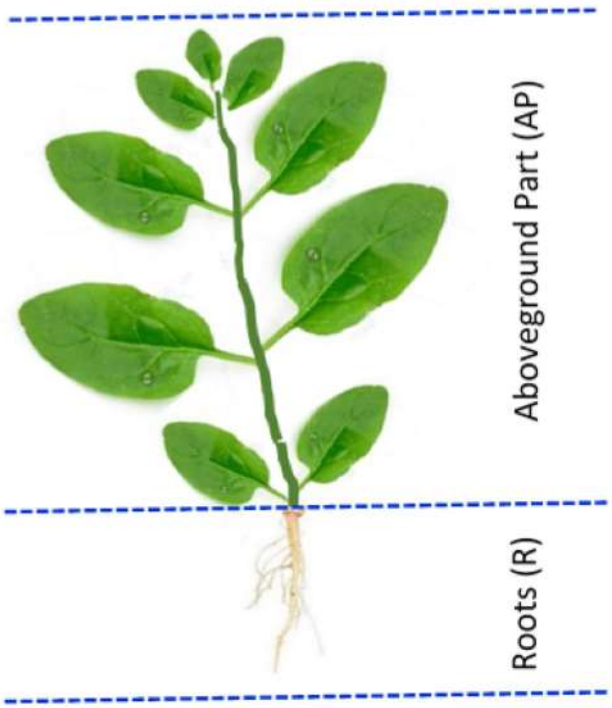

Fig. 2 Parts of the spinach plant

Statistical analysis

All elemental concentrations are reported in mg/plant or μg/plant. The raw data were checked for normality and homoscedasticity (constant variance) before the parametric test, e.g., analysis of variance (ANOVA). The one-way analysis of variance (ANOVA) was performed followed by a Fisher's pairwise comparison among the treatments for the growth parameters and the uptake of elements. The results are presented as the mean ± SD (standard deviation, $n = 3$). All statistical analysis was performed on Minitab (version 19). Significance was determined based on whether the p values are < 0.05 or not.

Results and discussion

Particle characterization

The average particle size of NZVI was 41.18 ± 16.16 nm ($n = 80$) and MZVI particles were < 44 µm, and both the particles were spherical (Fig. 6a-c). The SEM–EDS data for MZVI (Fig. 6d) showed 96.35% Fe and 1.31% oxygen and NZVI (Fig. 6e) showed 71.87% Fe and 13.67% oxygen, indicating the presence of elemental Fe (Fe^0) in the particle and an iron oxide layer around the core (core–shell structure). The percent oxygen in the NZVI conforms with Krajangpan et al. (2012) who reported it as 15.66%. A 2–4-nm-thin oxide layer on NZVI particles was reported by others [35, 36]. The presence of a very low amount (0.51%) of Na was observed in the NZVI. Sodium (Na) was possibly left behind as a residue from sodium borohydride ($NaBH_4$) used in the NZVI synthesis process.

Spinach growth

Spinach germination

Spinach seed germination in Petri dishes started after 2 days, and the percent of seed germination varied from 80 to 100%. Spinach plants having similar germination time and growth were transferred to the hydroponic solution.

Table 2 Dry biomass of root and the aboveground part of a spinach plant exposed to NZVI, MZVI, and $FeSO_4$

Treatment	Root (mg/plant)	Aboveground part (mg/plant)
Control	6.0 ± 4.2d	30.5 ± 17.6d
NZVI 11	16.5 ± 5.6cd	142.6 ± 52.9cd
NZVI 55	78.1 ± 27.1ab	674.5 ± 142.5a
NZVI 110	31.3 ± 19.1cd	342.9 ± 209.0bcd
MZVI 11	23.8 ± 9.0cd	198.6 ± 79.2cd
MZVI 55	38.2 ± 20.8bcd	333.0 ± 209.7bcd
MZVI 110	87.7 ± 63.4a	610.9 ± 406.1ab
$FeSO_4$ 11	27.4 ± 7.9cd	182.5 ± 54.3cd
$FeSO_4$ 55	51.0 ± 24.4abc	427.3 ± 229.9abc
$FeSO_4$ 110	18.1 ± 15.8cd	113.4 ± 88.8cd

Treatments were as follows: (i) Control: all nutrients (no Fe), (ii) all nutrients (no Fe) + NZVI 11 mg/L (iii) all nutrients (no Fe) + NZVI 55 mg/L (iv) all nutrients (no Fe) + NZVI 110 mg/L (v) all nutrients (no Fe) + MZVI 11 mg/L (vi) all nutrients (no Fe) + MZVI 55 mg/L (vii) all nutrients (no Fe) + MZVI 110 mg/L (viii) all nutrients (no Fe) + $FeSO_4$ 11 mg/L (ix) all nutrients (no Fe) + $FeSO_4$ 55 mg/L (x) all nutrients (no Fe) + $FeSO_4$ 110 mg/L. Except for the control, the rest of the treatments were supplemented with the modified Hoagland solution containing all elements but Fe. Differences were determined by a one-way ANOVA followed by Fisher's pairwise comparisons ($p < 0.05$). Different letters in the same column indicate significant differences between different treatments. The values for the control (with no Fe) treatment should be ignored as there was negligible plant biomass for spinach in this treatment

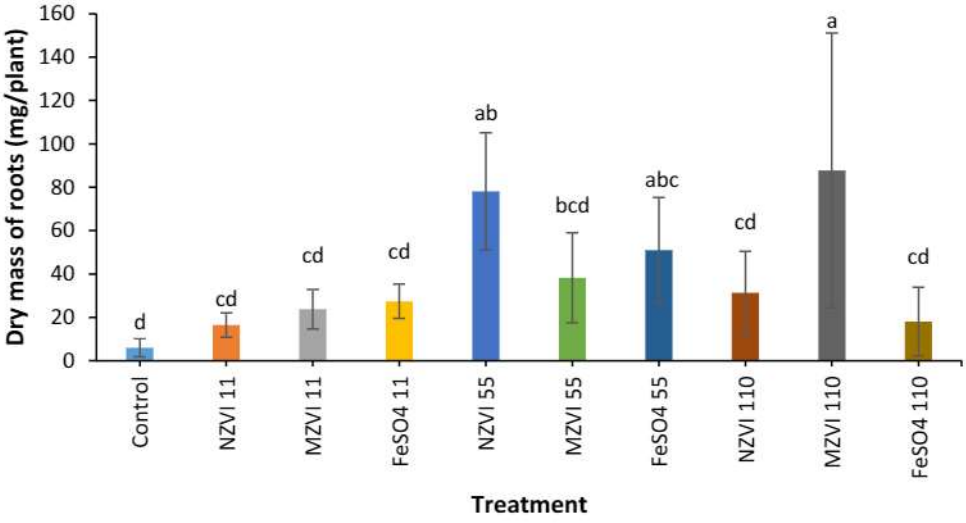

Fig. 3 Dry biomass of roots of a spinach plant exposed to NZVI, MZVI, and $FeSO_4$. Treatments were as follows: (i) Control: all nutrients (no Fe), (ii) all nutrients (no Fe) + NZVI 11 mg/L (iii) all nutrients (no Fe) + NZVI 55 mg/L (iv) all nutrients (no Fe) + NZVI 110 mg/L (v) all nutrients (no Fe) + MZVI 11 mg/L (vi) all nutrients (no Fe) + MZVI 55 mg/L (vii) all nutrients (no Fe) + MZVI 110 mg/L (viii) all nutrients (no Fe) + $FeSO_4$ 11 mg/L (ix) all nutrients (no Fe) + $FeSO_4$ 55 mg/L (x) all nutrients (no Fe) + $FeSO_4$ 110 mg/L. Except for the control, the rest of the treatments were supplemented with the modified Hoagland solution containing all elements but Fe. Differences were determined by a one-way ANOVA followed by Fisher's pairwise comparison ($p < 0.05$). Different letters above bars indicate significant differences between different treatments. The values for the control (with no Fe) treatment should be ignored as there was negligible plant biomass for spinach in this treatment

Spinach dry biomass

After drying at 65 °C, the roots (R) and the aboveground part (AP) of the plants were weighed. Root (R) and aboveground part (AP) biomass of spinach grown in all the reactors were recorded separately by first combining biomass of the three plants from each reactor and subsequently averaging them (on per plant basis) (Fig. 3 and 4 and Table 2).

Aboveground part The aboveground part of the spinach plant consisted of the edible stem and leaves. The biomass data (Fig. 4 and Table 2) indicate that the plants in the NZVI 55 treatment (674.5 mg biomass/plant) fared better compared to the other treatments. The biomass was statistically significantly better (one-way ANOVA, $p<0.05$) than the rest of the treatments except that obtained with MZVI 110 and $FeSO_4$ 55. For the aboveground biomass, NZVI and $FeSO_4$ were found to enhance the growth of spinach at the medium concentration (55 mg/L), whereas MZVI yielded a better response at the highest concentration (110 mg/L) (Table 2). Again, NZVI 55, MZVI-110, and $FeSO_4$-55 treatments were compared. The increase was ~ 1.57-fold for NZVI-55 compared to $FeSO_4$-55, and there was a 1.43-fold biomass increase in MZVI-110 compared to $FeSO_4$-55. NZVI 55-treated plants had ~ 1.10-fold more biomass compared to MZVI 110.

Roots The dry mass of spinach roots varied depending on the treatment (MZVI, NZVI, or $FeSO_4$). Ferrous sulfate is an ionic counterpart for NZVI and MZVI particles and is typically used for hydroponic media. For MZVI, the root mass increased gradually as the concentration increased. For NZVI, the highest root biomass was recorded at 55 mg/L treatment and biomass was less at the highest NZVI concentration. A trend as in NZVI was observed for $FeSO_4$ with the highest concentration of $FeSO_4$ negatively affecting root biomass growth. At the lowest iron concentration (11 mg Fe/L), there was no significant difference in root biomass development among the three treatments (one-way ANOVA, $p>0.05$). At 55 mg Fe/L, biomass increased in all treatments compared to 11 mg Fe/L treatments. However, only NZVI treatment exhibited statistically significant increase in biomass growth (one-way ANOVA, $p<0.05$). As the iron concentration increased (to 110 mg Fe/L), the root biomass growth decreased in NZVI- and $FeSO_4$-treated plants, but MZVI-treated plants had a significant jump in biomass growth (Fig. 3, Table 2).

The applications of 110 mg/L MZVI (MZVI-110), 55 mg/L NZVI (NZVI-55), and 55 mg/L $FeSO_4$ ($FeSO_4$-55) resulted in statistically similar (one-way ANOVA, $p>0.05$) root biomass growth and so we found them worth comparing further. The mean dry masses of roots were 78.1 mg for NZVI-55, 87.7 mg for MZVI-110, and 51.0 mg for

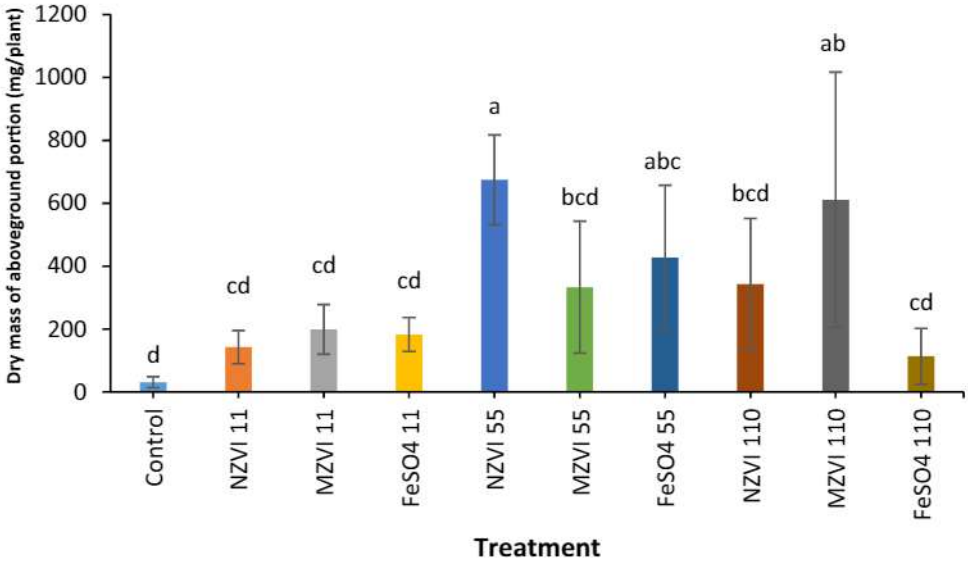

Fig. 4 Dry biomass of aboveground portion of a spinach plant exposed to NZVI, MZVI, and $FeSO_4$. Treatments were as follows: (i) Control: all nutrients (no Fe), (ii) all nutrients (no Fe) + NZVI 11 mg/L (iii) all nutrients (no Fe) + NZVI 55 mg/L (iv) all nutrients (no Fe) + NZVI 110 mg/L (v) all nutrients (no Fe) + MZVI 11 mg/L (vi) all nutrients (no Fe) + MZVI 55 mg/L (vii) all nutrients (no Fe) + MZVI 110 mg/L (viii) all nutrients (no Fe) + $FeSO_4$ 11 mg/L (ix) all nutrients (no Fe) + $FeSO_4$ 55 mg/L (x) all nutrients (no Fe) + $FeSO_4$ 110 mg/L. Except for the control, the rest of the treatments were supplemented with the modified Hoagland solution containing all elements but Fe. Differences were determined by a one-way ANOVA followed by Fisher's pairwise comparison ($p<0.05$). Different letters above bars indicate significant differences between different treatments. The values for the control (with no Fe) treatment should be ignored as there was negligible plant biomass for spinach in this treatment

$FeSO_4$-55 treatments. Given that root biomass from the control treatment was so less (the control plants were not healthy due to lack of iron), we decided not to compare our results with the control but compared NZVI and MZVI treatments with treatments where the conventional iron fertilizer $FeSO_4$ was used. NZVI-55 treatment yielded ~ 1.53-fold increase over $FeSO_4$-55, and MZVI-110 yielded ~ 1.72-fold increase over $FeSO_4$-55 mg/L.

Macro- and micronutrient analysis

All the macroelements and microelements in spinach plants were analyzed and reported here (Tables 3 and 4) as mg or µg per plant [17]. Again, the control group (the plants grown without iron in the growth solution) was ignored as there was not enough biomass in the spinach from this group.

Table 3 Uptake of Fe, Zn, Mn, Cu, and B by spinach plant exposed to NZVI, MZVI, and $FeSO_4$

Treatment	Fe µg/plant	Zn	Mn	Cu	B
Control	1.25 ± 0.68d	5.00 ± 2.67c	6.22 ± 3.34d	0.81 ± 0.48c	2.34 ± 1.23c
NZVI 11	16.12 ± 7.20cd	42.05 ± 20.49b	68.49 ± 38.44bcd	3.46 ± 1.61b	14.11 ± 4.20c
NZVI 55	84.66 ± 14.48a	76.95 ± 8.35a	154.20 ± 34.02a	5.64 ± 0.39a	96.24 ± 40.48a
NZVI 110	47.39 ± 8.64bc	61.82 ± 13.45ab	145.86 ± 54.23a	4.93 ± 0.84ab	70.32 ± 38.80ab
MZVI 11	18.50 ± 7.77cd	58.40 ± 32.29ab	107.35 ± 66.11ab	4.19 ± 1.66ab	14.42 ± 3.89c
MZVI 55	49.34 ± 29.87abc	59.71 ± 24.95ab	107.21 ± 41.84ab	4.75 ± 2.16ab	21.79 ± 14.46c
MZVI 110	73.76 ± 45.38ab	65.61 ± 5.68ab	125.55 ± 24.42ab	5.44 ± 0.66ab	36.54 ± 21.44bc
$FeSO_4$ 11	22.89 ± 18.63cd	41.59 ± 7.36b	71.86 ± 3.36bc	3.91 ± 0.33ab	14.94 ± 3.75c
$FeSO_4$ 55	49.92 ± 25.67abc	48.76 ± 23.98ab	73.75 ± 44.95bc	4.24 ± 1.68ab	32.27 ± 16.31c
$FeSO_4$ 110	21.14 ± 17.03cd	9.43 ± 9.27c	9.35 ± 8.92cd	1.15 ± 1.03c	8.08 ± 6.80c

Treatments were as follows: (i) Control: all nutrients (no Fe), (ii) all nutrients (no Fe) + NZVI 11 mg/L (iii) all nutrients (no Fe) + NZVI 55 mg/L (iv) all nutrients (no Fe) + NZVI 110 mg/L (v) all nutrients (no Fe) + MZVI 11 mg/L (vi) all nutrients (no Fe) + MZVI 55 mg/L (vii) all nutrients (no Fe) + MZVI 110 mg/L (viii) all nutrients (no Fe) + $FeSO_4$ 11 mg/L (ix) all nutrients (no Fe) + $FeSO_4$ 55 mg/L (x) all nutrients (no Fe) + $FeSO_4$ 110 mg/L. Except for the control, the rest of the treatments were supplemented with the modified Hoagland solution containing all elements but Fe. Differences were determined by a one-way ANOVA followed by Fisher's pairwise comparisons ($p < 0.05$). Different letters in the same column indicate significant differences between different treatments. The values for the control (with no Fe) treatment should be ignored as there was negligible plant biomass for spinach in this treatment

Table 4 Uptake of P, K, S, Ca, Mg and Na by spinach plant exposed to NZVI, MZVI, and $FeSO_4$

Treatment	P mg/plant	K	S	Ca	Mg	Na
Control	0.18 ± 0.13c	1.78 ± 0.98e	0.09 ± 0.06e	0.43 ± 0.24d	0.19 ± 0.10d	0.04 ± 0.02b
NZVI 11	1.20 ± 0.55bc	13.15 ± 5.44de	0.51 ± 0.21cde	1.59 ± 0.69 cd	1.10 ± 0.38 cd	0.05 ± 0.02b
NZVI 55	4.59 ± 1.25a	68.55 ± 17.07a	2.21 ± 0.58a	4.62 ± 0.88ab	5.05 ± 1.34a	0.31 ± 0.07a
NZVI 110	2.55 ± 0.84abc	37.08 ± 14.08bcd	1.39 ± 0.47abc	3.46 ± 1.28abc	3.05 ± 1.46abc	0.39 ± 0.15a
MZVI 11	1.70 ± 0.80bc	18.72 ± 9.53cde	0.78 ± 0.34cde	1.68 ± 0.12 cd	1.72 ± 0.81 cd	0.14 ± 0.09b
MZVI 55	2.74 ± 1.79ab	34.27 ± 23.16bcd	1.18 ± 0.71bcd	2.36 ± 1.21 cd	2.45 ± 1.58bcd	0.16 ± 0.05b
MZVI 110	4.54 ± 3.31a	57.36 ± 38.95ab	2.07 ± 1.33ab	4.89 ± 3.18a	4.29 ± 2.82ab	0.31 ± 0.09a
$FeSO_4$ 11	1.45 ± 0.39bc	16.44 ± 4.92cde	0.65 ± 0.15cde	1.61 ± 0.37 cd	1.36 ± 0.32 cd	0.07 ± 0.04b
$FeSO_4$ 55	3.12 ± 1.49ab	44.82 ± 25.05abc	1.36 ± 0.66abcd	2.44 ± 1.20bcd	2.95 ± 1.47abc	0.14 ± 0.04b
$FeSO_4$ 110	0.76 ± 0.60bc	8.83 ± 8.80de	0.37 ± 0.32de	0.62 ± 0.50d	0.66 ± 0.68d	0.06 ± 0.06b

Treatments were as follows: (i) Control: all nutrients (no Fe), (ii) all nutrients (no Fe) + NZVI 11 mg/L (iii) all nutrients (no Fe) + NZVI 55 mg/L (iv) all nutrients (no Fe) + NZVI 110 mg/L (v) all nutrients (no Fe) + MZVI 11 mg/L (vi) all nutrients (no Fe) + MZVI 55 mg/L (vii) all nutrients (no Fe) + MZVI 110 mg/L (viii) all nutrients (no Fe) + $FeSO_4$ 11 mg/L (ix) all nutrients (no Fe) + $FeSO_4$ 55 mg/L (x) all nutrients (no Fe) + $FeSO_4$ 110 mg/L. Except for the control, the rest of the treatments were supplemented with the modified Hoagland solution containing all elements but Fe. Differences were determined by a one-way ANOVA followed by Fisher's pairwise comparisons ($p < 0.05$). Different letters in the same column indicate significant differences between different treatments. The values for the control (with no Fe) treatment should be ignored as there was negligible plant biomass for spinach in this treatment

Iron uptake

The uptake of Fe by plants dosed with different concentrations of NZVI, MZVI, and $FeSO_4$ was monitored. The roots did not yield enough dry mass; it was difficult to analyze the elemental concentrations in the roots for all the treatments. Consequently, we ignored the root uptake of elements and the following discussion is only for the Fe uptake by the aboveground part of the spinach plants (Table 3 and Fig. 5). The uptake of iron (Fe) was found to be affected by the type of treatment (one-way ANOVA, $p < 0.05$). The mean uptake of Fe was found to be higher (84.66 µg/plant) in NZVI-55 which is significantly different from NZVI-11, NZVI-110, MZVI-11, $FeSO_4$-11, and $FeSO_4$-110 (one-way ANOVA, $p < 0.05$). The second highest uptake (73.76 µg/plant) was observed with MZVI-110 treatment; however, the variability was high. NZVI-110, MZVI-55, and $FeSO_4$-55 fared almost equally well, and there were no significant differences among these three treatments (one-way ANOVA, $p > 0.05$). Based on the Fe uptake data, we can still say that the spinach dosed with NZVI-55 showed a 1.70-fold increase over $FeSO_4$ 55-dosed spinach treated with the same iron concentration (55 mg/L). NZVI-55 showed a 1.15-fold increase over MZVI-110. The uptake was the highest when plants were supplemented with NZVI at 55 mg/L. At the higher dose, NZVI might have hampered the growth of plants because of the higher concentration of Fe^{3+} ions available from the nanomaterials [37]. Ma et al. (2013) [37] reported that NZVI gets oxidized to Fe^{2+} ions which are further oxidized to its less soluble form (Fe^{3+}) by the oxidizing agents that plant root exudes; thus, a layer of an insoluble Fe^{3+} compound is formed on the root surface. This insoluble compound along with NZVI particles could block the membrane pores and appreciably reduce the efficacy of root uptake of water and nutrients [37]. NZVI-55 and MZVI-110 treatments caused profuse biomass growth in spinach, and enhanced uptake was associated with the higher biomass of the plants. NZVI at 55 mg/L and MZVI at 110 mg/L elicited the same response from plants in terms of iron uptake. Others have reported that the smaller particle size of NZVI increases its chemical reactivity and thus increases the availability of iron ions [38–40].

Phosphorus, potassium, sulfur, calcium, and magnesium uptake

Besides iron, the uptake patterns of different macroelements were also analyzed in the plants treated with NZVI, MZVI, and $FeSO_4$ (Table 4). The total uptake of the elements varied among the treatments. The uptake of phosphorus (P) was the highest (4.59 mg/plant) with the NZVI-55 treatment which was significantly different from NZVI-11, MZVI-11,

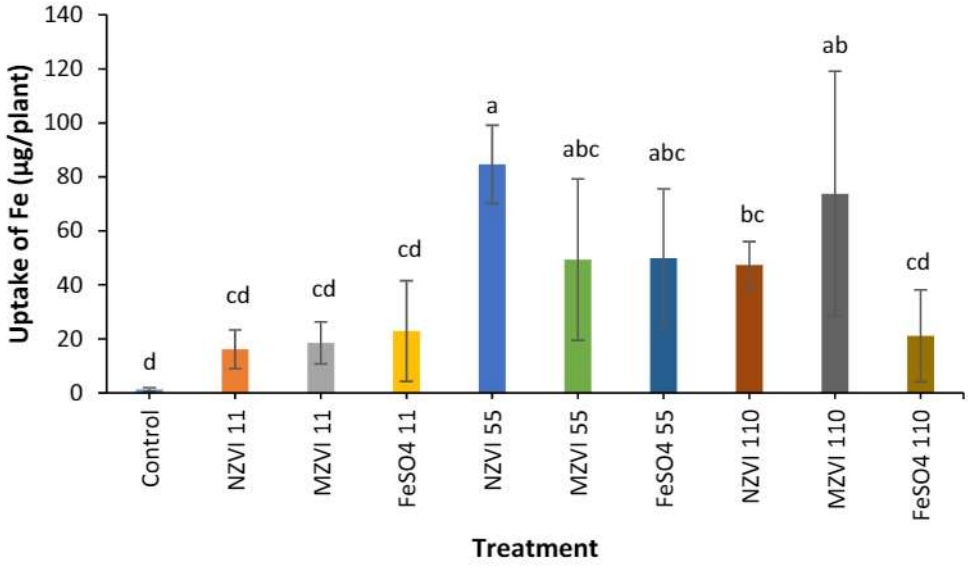

Fig. 5 Uptake of Fe by a spinach plant exposed to NZVI, MZVI and $FeSO_4$. Treatments were as follows: (i) Control: all nutrients (no Fe), (ii) all nutrients (no Fe) + NZVI 11 mg/L (iii) all nutrients (no Fe) + NZVI 55 mg/L (iv) all nutrients (no Fe) + NZVI 110 mg/L (v) all nutrients (no Fe) + MZVI 11 mg/L (vi) all nutrients (no Fe) + MZVI 55 mg/L (vii) all nutrients (no Fe) + MZVI 110 mg/L (viii) all nutrients (no Fe) + $FeSO_4$ 11 mg/L (ix) all nutrients (no Fe) + $FeSO_4$ 55 mg/L (x) all nutrients (no Fe) + $FeSO_4$ 110 mg/L. Except for the control, the rest of the treatments were supplemented with the modified Hoagland solution containing all elements but Fe. Differences were determined by a one-way ANOVA followed by Fisher's pairwise comparison ($p < 0.05$). Different letters above bars indicate significant differences between different treatments. The values for the control (with no Fe) treatment should be ignored as there was negligible plant biomass for spinach in this treatment

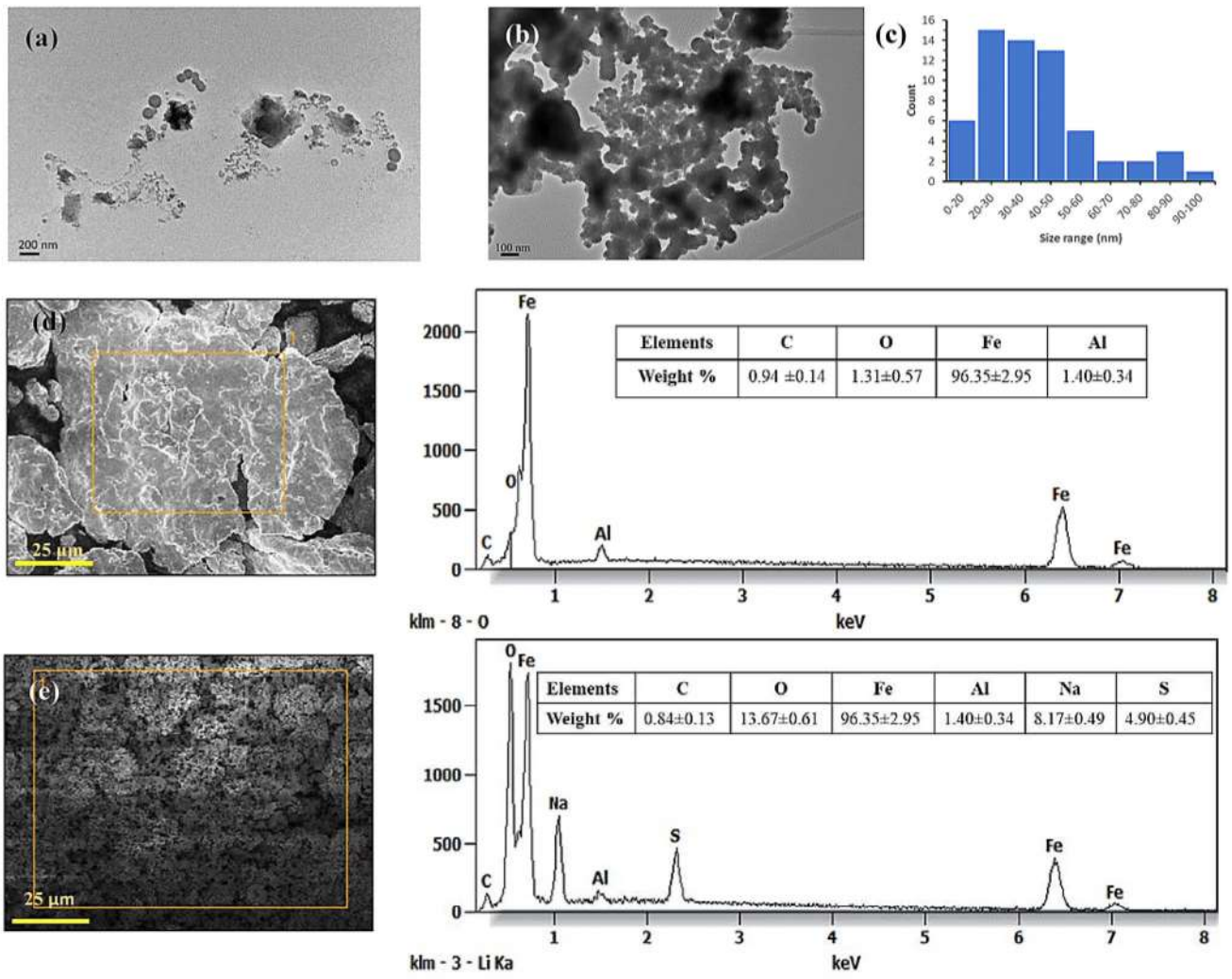

Fig. 6 **(a)** TEM micrograph of MZVI particles; **(b)** TEM micrograph of NZVI particles; **(c)** particle size distribution of NZVI; **(d)** EDS spectrum of MZVI; and **(e)** EDS spectrum of NZVI

$FeSO_4$-11, and $FeSO_4$-110 (one-way ANOVA, $p < 0.05$). The MZVI-110 treatment also enhanced the uptake of P (4.54 mg/plant) and matched the P uptake in the NZVI-55 treatment (one-way ANOVA, $p > 0.05$). The treatment with $FeSO_4$ at 55 mg/L has no statistically significant differences with NZVI-55 and MZVI-110 treatments (one-way ANOVA, $p > 0.05$).

The uptake of potassium (K) was the highest (68.55 mg/plant) for the NZVI-55 treatment followed by the MZVI-110 (57.36 mg/plant) and the $FeSO_4$-55 (44.82 mg/plant) treatments. These three treatments did not differ significantly from one another. However, the NZVI-55 treatment was found to be significantly different from the NZVI-11, NZVI-110, MZVI-11, MZVI-55, $FeSO_4$-11, and $FeSO_4$-110 treatments (one-way ANOVA, $p < 0.05$). Sulfur uptake was also the highest (2.21 mg/plant) for the NZVI-55 treatment followed by the MZVI-110 (2.07 mg/plant) and NZVI-110 (1.39 mg/plant) treatments, and they significantly differed from each other (one-way ANOVA, $p < 0.05$).

Calcium (Ca) did not follow the pattern of the other elements. The highest Ca uptake (4.89 mg/plant) was observed in the spinach plants treated with MZVI-110. The Ca uptake was 4.62 mg/plant (second highest) for NZVI-55 and 3.46 mg/plant (third highest) for NZVI-110-treated plants. Magnesium (Mg) uptake was the highest (5.05 mg/plant) in the plants treated with NZVI-55 followed by MZVI-110 (4.29 mg/plant) and NZVI-110 (3.05 mg/plant). Again, Na uptake by plants dosed with NZVI-110, NZVI-55, and MZVI-110 significantly differed from the rest of the treatments (one-way ANOVA, $p < 0.05$). The high Na amount in NZVI-treated plants should be viewed with the caveat that the NZVI particles were synthesized using the sodium borohydride method (Fig. 6e) and there was ~ 8.17% Na (from EDS analysis) in the synthesized nanoparticles.

Zinc, manganese, copper, and boron uptake

The uptake of zinc (Zn), manganese (Mn), copper (Cu), and boron (B) was affected significantly by the type treatment (one-way ANOVA, $p < 0.05$) (Table 3). The highest uptake (76.95 µg/plant) of Zn was observed in the plants treated with NZVI-55 followed by those treated with MZVI-110 (65.61 µg/plant) and NZVI-110 (61.82 µg/plant). Manganese (Mn) uptake was the maximum in the plants dosed with NZVI-55 (154.20 µg/plant) followed by NZVI-110 (145.86 µg/plant) and MZVI-110 (125.55 µg/plant). The NZVI-55 and NZVI-110 treatments are significantly different from the NZVI-11, $FeSO_4$-11, $FeSO_4$-55, and $FeSO_4$-110 treatments (one-way ANOVA, $p < 0.05$). The highest copper (Cu) uptake (5.64 µg/plant) was by the NZVI-55 followed by the MZVI-110 (5.44 µg/plant) and NZVI-11 (4.93 µg/plant) treatment.

Boron (B) uptake was the highest in the plants treated with NZVI-55 followed by NZVI-110 and MZVI-110. The NZVI-55 and NZVI-110 treatments were significantly different from the rest of the treatments (one-way ANOVA, $p < 0.05$). Excess B in the plants (dosed with NZVI) might have come from the NZVI used for the experiment. The NZVI used in this experiment was synthesized using the sodium borohydride method, and boron probably remained in NZVI as a residue.

In the present study, the plant uptake of macro- and micronutrients was found to be modulated based on the type of iron (nano, micro, or ionic), and the dose (mg Fe/L) to which the plants were exposed. NZVI at 55 mg/L was seen to increase the uptake of most of the elements in spinach. MZVI at 110 mg/L was found to increase the uptake of Zn, Mn, Cu, and B that was comparable to when NZVI-55 was used. Others reported that spinach plants treated with FeS_2 nanoparticles exhibited increased Ca, Mn, and Zn accumulations [29]. That the uptake was high with the application of NZVI-55 could be directly associated with the higher yield of biomass. Plants treated with NZVI-55 had higher biomass compared to other treatments. For MZVI, the plant uptake of mineral elements increased with the increase MZVI dose, but this trend was not observed for NZVI and $FeSO_4$. For NZVI and $FeSO_4$ treatments, the trend showed an initial increase in total uptake followed by a decrease in the uptake. At higher concentrations (110 mg/L), $FeSO_4$ and NZVI might have caused phytotoxicity, resulting in reduced yield of biomass which in turn decreased the total uptake of elements. Others have also reported that iron and iron oxide nanoparticles promote growth at low concentrations and start showing toxic effects beyond a certain higher concentration [1, 38]. The high biomass growth with NZVI-55 in our work is congruent with the work of Guha et al. (2018) [41] who reported increased photosynthetic pigment, and hence, biomass growth in the seedlings of rice cultivar *Oryza sativa* cv. Gobindabhog primed with low doses of NZVI (< 80 mg/L). In the same study, a higher concentration (160 mg/L) of NZVI caused oxidative stress in rice that led to root tissue damage [41]. Working with *Capsicum annum*, others have demonstrated that even a very low concentration of iron nanoparticles (0.05 mM or 1.3 mg/L) promoted plant growth by influencing leaf organization and increasing chloroplast number and grana stacking [1]. The iron nanoparticles were found to regulate the organization of vascular bundles at that concentration. They also reported that the nanoparticles were harmful to the plant at a higher concentration [1] which is in agreement with our findings. In a spinach study with nano-Fe_2O_3, higher concentrations (100, 150, and 200 mg/L) were used, and enhanced root and stem growths were observed in a dose-dependent manner [25]. They attributed the enhanced growth to the uptake of iron via roots and subsequent translocation to the various parts of the plant [25]. Rui et al. (2016) [39] used iron oxide (Fe_2O_3) nanoparticles in peanut (*Arachis hypogaea*) and observed increased root length, plant height, biomass, and SPAD values that were comparable to Fe-EDTA application. Generally, iron or iron oxide nanomaterials do not pose toxic effects at concentrations below ~ 100 mg/L but start showing toxicity beyond 100 mg/L [38]. Ma et al. (2013) [37], however, reported reduced growth and low biomass in *Typha latifolia* and hybrid poplar (*Populous deltoids* × *Populous nigra*) at much higher NZVI concentrations (> 200 mg/L). Similarly, ryegrass (*Lolium perenne*), barley (*Hordeum vulgare*), and flax (*Linum usitatissimum*) showed inhibitory effects only at an NZVI concentration of 250 mg/L [42].

From our study with spinach, it is very evident that the presence of iron nanoparticles and microparticles affected the plant uptake of iron and other elements. The presence of the particles enhanced biomass growth and affected the uptake of iron (Fe) and other elements (P, K, S, Ca, Mg, Zn, Fe, Mn, and Cu) in a positive way. Optimal biofortification of plants with Fe was achieved with 55 mg/L NZVI, and the uptake of other elements was positively affected in the process. The MZVI particles (microscale counterpart of NZVI) also performed well but at a higher concentration (110 mg/L).

The plant uptake of Fe from NZVI as well as from MZVI indicates that plants were able to use Fe using some Fe acquisition strategies. The oxidation of NZVI/MZVI and its subsequent dissolution might have provided the plant with sufficient ionic iron (Fe^{2+}) in the solution needed for growth [43]. Core–shell (Fe/Fe_xO_y/FeOOH)-structured NZVI was reported to release 1.7 mg/L of iron ions in the aqueous phase (when 250 mg NZVI/L was used) [33]. However, it is not clear as to how NZVI helped in the increased uptake of other nutrient elements in spinach. In a previous study with

Arabidopsis thaliana, NZVI was found to enhance root elongation by triggering OH• radical-induced cell wall loosening (degradation of polysaccharides) which in turn increased endocytosis in root cells [44]. In another study, NZVI was reported to trigger high plasma membrane H^+-ATPase activity in *Arabidopsis thaliana* which resulted in a decrease in apoplastic pH, an increase in leaf area, and also wider stomatal aperture [45]. They attributed these phenomena to AHA2 gene (plasma membrane H^+-ATPase gene) in the roots and leaves [45]. This gene is typically involved in rhizosphere acidification and the stomatal opening of leaves [45].

Conclusions

In this study, the feasibility of using nanoscale zero-valent iron (NZVI) and microscale zero-valent iron (MZVI) as fortificants (or nano/micro fertilizers) was tested using spinach as the model plant. The experimental results suggest that NZVI and MZVI can be used as fortificants for spinach. The dose of NZVI and MZVI would be an important factor for biofortification. Spinach produced more biomass when treated with NZVI at 55 mg/L and MZVI at 110 mg/L. NZVI (55 mg Fe/L) increased the aboveground biomass (the edible part) ~ 1.10-fold compared to MZVI (110 mg Fe/L) and ~ 1.57-fold compared to $FeSO_4$ (55 mg Fe/L). There was a 1.15-fold increase in iron uptake in the plants treated with NZVI (55 mg Fe/L) compared to MZVI (110 mg Fe/L), and the increase was ~ 1.70-fold compared to $FeSO_4$ (55 mg Fe/L) treatment. Both NZVI and MZVI promoted the uptake of some important plant macro- and microelements. While additional research would be required, we can say that nanoscale and microscale iron particles can potentially be used as fertilizers for crop production. Further research will be needed to get more insight into the mode, dose, and time of application of NZVI and MZVI as fortificants in agricultural fields.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Yuan J, Chen Y, Li H, Lu J, Zhao H, Liu M, Nechitaylo GS, Glushchenko NN (2018) New insights into the cellular responses to iron nanoparticles in Capsicum annuum. Sci Rep 8(1):3228
2. Briat JF, Dubos C, Gaymard F (2015) Iron nutrition, biomass production, and plant product quality. Trends Plant Sci 20(1):33–40
3. Morgan JB, Connolly EL (2013) Plant-soil interactions: nutrient uptake. Nat Educ Knowl 4(8):2
4. Broadley M, Brown P, Cakmak I, Rengel Z, Zhao F (2021) Function of nutrients: Micronutrients, Marschner's mineral nutrition of higher plants, 3rd Edition 191–248
5. Geissler C, Singh M (2011) Iron, meat and health. Nutrients 3(3):283–316
6. Bailey RL, West KP Jr, Black RE (2015) The epidemiology of global micronutrient deficiencies. Ann Nutr Metab 66:22–33
7. Boccio JR, Iyengar V (2003) Iron deficiency: causes, consequences, and strategies to overcome this nutritional problem. Biol Trace Elem Res 94(1):1
8. Johnson-Wimbley TD, Graham DY (2011) Diagnosis and management of iron deficiency anemia in the 21st century. Ther Adv Gastroenterol 4(3):177–184
9. World Health Organization (WHO) (2021) The Global Health Observatory, World Health Organization, Geneva, Switzerland, 2021. Available at https://www.who.int/data/gho/data/indicators/indicator-details/GHO/. Accessed May 2021
10. Gomez-Galera S, Rojas E, Sudhakar D, Zhu C, Pelacho AM, Capell T, Christou P (2010) Critical evaluation of strategies for mineral fortification of staple food crops. Transgenic Res 19(2):165–180
11. White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182(1):49
12. Duran P, Acuna JJ, Jorquera MA, Azcon R, Borie F, Cornejo P, Mora ML (2013) Enhanced selenium content in wheat grain by co-inoculation of selenobacteria and arbuscular mycorrhizal fungi: a preliminary study as a potential Se biofortification strategy. J Cereal Sci 57(3):275–280
13. Jeong J, Guerinot ML (2008) Biofortified and bioavailable: the gold standard, for plant-based diets. Proc Natl Acad Sci USA 105(6):1777–1778
14. Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH (2014) Biofortification: a new tool to reduce micronutrient malnutrition. Improving Diets and Nutrition: Food-Based Approaches 202–215
15. Ramzani PMA, Khalid M, Naveed M, Ahmad R, Shahid M (2016) Iron biofortification of wheat grains through integrated use of organic and chemical fertilizers in pH affected calcareous soil. Plant Physiol Biochem 104:284–293
16. Lucena C, Romera FJ, Rojas CL, Garcia M, Alcantara E, Perez-Vicente R (2007) Bicarbonate blocks the expression of several genes involved in the physiological responses to Fe deficiency of Strategy I plants. Funct Plant Biol 34(11):1002–1009
17. Almeelbi T, Bezbaruah A (2014) Nanoparticle-sorbed phosphate: iron and phosphate bioavailability studies with spinacia oleracea and selenastrum capricornutum. ACS Sustain Chem Eng 2(7):1625–1632
18. Ortega-Blu R, Molina-Roco M (2007) Comparison between sulfates and chelated compounds as sources of zinc and iron in calcareous soils. Agrociencia 41(5):491–502
19. Aciksoz SB, Yazici A, Ozturk L, Cakmak I (2011) Biofortification of wheat with iron through soil and foliar application of nitrogen and iron fertilizers. Plant Soil 349(1–2):215–225

20. Sharma S, Chandra S, Kumar A, Bindraban P, Saxena AK, Pande V, Pandey R (2019) Foliar application of iron fortified bacteriosiderophore improves growth and grain Fe concentration in wheat and soybean. Indian J Microbiol 59(3):344–350
21. Zou CQ, Du YF, Rashid A, Ram H, Savasli E, Pieterse PJ, Ortiz-Monasterio I, Yazici A, Kaur C, Mahmood K, Singh S, Le Roux MR, Kuang W, Onder O, Kalayci M, Cakmak I (2019) Simultaneous biofortification of wheat with zinc, iodine, selenium, and iron through foliar treatment of a micronutrient cocktail in six countries. J Agric Food Chem 67(29):8096–8106
22. Jalal A, Shah S, Teixeira MCM, Khan A, Shah T, Ilyas M, Rosa PAL (2020) Agro-biofortification of zinc and iron in wheat grains. Gesunde Pflanzen 72:227–236
23. Ramzan Y, Hafeez MB, Khan S, Nadeem M, Saleem ur R, Batool S, Ahmad J (2020) Biofortification with zinc and iron improves the grain quality and yield of wheat crop. Int J Plant Prod 10
24. Perez-de-Luque A, Hermosin MC (2013) Nanotechnology and its use in agriculture. In: Bagchi D, Bagchi M, Moriyama H, Shahidi F (eds) Biotechnology: A Revolution in Food. Biomedical and Health Sciences, Wiley-Blackwell, West Sussex, UK, pp 383–398
25. Jeyasubramanian K, Gopalakrishnan Thoppey UU, Hikku GS, Selvakumar N, Subramania A, Krishnamoorthy K (2016) Enhancement in growth rate and productivity of spinach grown in hydroponics with iron oxide nanoparticles. RSC Adv 6(19):15451–15459
26. Ghafariyan MH, Malakouti MJ, Dadpour MR, Stroeve P, Mahmoudi M (2013) Effects of magnetite nanoparticles on soybean chlorophyll. Environ Sci Technol 47(18):10645–10652
27. Delfani M, Firouzabadi MB, Farrokhi N, Makarian H (2014) Some physiological responses of black-eyed pea to iron and magnesium nanofertilizers. Commun Soil Sci Plant Anal 45(4):530–540
28. Food and Agriculture Organization of the United Nations (FAO) (2021) FAOSTAT Crops, Food and Agriculture Organization of the United Nations, Rome, Italy, 2020. Available at http://www.fao.org/faostat/en/#data/QC. Accessed May 2021
29. Srivastava G, Das CK, Das A, Singh SK, Roy M, Kim H, Sethy N, Kumar A, Sharma RK, Philip D, Das M (2014) Seed treatment with iron pyrite (FeS2) nanoparticles increases the production of spinach. RSC Adv 4(102):58495–58504
30. Almeelbi T, Bezbaruah A (2012) Aqueous phosphate removal using nanoscale zero-valent iron. J Nanopart Res 14(7):14
31. Jacob DL, Borchardt JD, Navaratnam L, Otte ML, Bezbaruah AN (2013) Uptake and translocation of Ti from nanoparticles in crops and wetland plants. Int J Phytorem 15(2):142–153
32. Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil (Circular 347). California Agricultural Experiment Station, California, USA
33. Trujillo-Reyes J, Majumdar S, Botez CE, Peralta-Videa JR, Gardea-Torresdey JL (2014) Exposure studies of core-shell Fe/Fe3O4 and Cu/CuO NPs to lettuce (Lactuca sativa) plants: are they a potential physiological and nutritional hazard? J Hazard Mater 267:255–263
34. Jones JB Jr (2001) Laboratory guide for conducting soil tests and plant analysis. CRC Press, New York, US
35. Krajangpan S, Kalita H, Chisholm BJ, Bezbaruah AN (2012) Iron nanoparticles coated with amphiphilic polysiloxane graft copolymers: dispersibility and contaminant treatability. Environ Sci Technol 46(18):10130–10136
36. Martin JE, Herzing AA, Yan WL, Li XQ, Koel BE, Kiely CJ, Zhang WX (2008) Determination of the oxide layer thickness in core-shell zerovalent iron nanoparticles. Langmuir 24(8):4329–4334
37. Ma X, Gurung A, Deng Y (2013) Phytotoxicity and uptake of nanoscale zero-valent iron (nZVI) by two plant species. Sci Total Environ 443:844–849
38. Gillispie EC, Taylor SE, Qafoku NP, Hochella MF (2019) Impact of iron and manganese nano-metal-oxides on contaminant interaction and fortification potential in agricultural systems - a review. Environ Chem 16(6):377–390
39. Rui MM, Ma CX, Hao Y, Guo J, Rui YK, Tang XL, Zhao Q, Fan X, Zhang ZT, Hou TQ, Zhu SY (2016) Iron oxide nanoparticles as a potential iron fertilizer for peanut (Arachis hypogaea). Front Plant Sci 7:10
40. Liu RQ, Zhang HY, Lal R (2016) Effects of stabilized nanoparticles of copper, zinc, manganese, and iron oxides in low concentrations on lettuce (Lactuca sativa) seed germination: nanotoxicants or nanonutrients? Water Air Soil Pollut 227(1):14
41. Guha T, Ravikumar KVG, Mukherjee A, Kundu R (2018) Nanopriming with zero valent iron (nZVI) enhances germination and growth in aromatic rice cultivar (Oryza sativa cv. Gobindabhog L.). Plant Physiol Biochem 127:403–413
42. El-Temsah YS, Joner EJ (2012) Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. Environ Toxicol 27(1):42–49
43. Kadar E, Rooks P, Lakey C, White DA (2012) The effect of engineered iron nanoparticles on growth and metabolic status of marine microalgae cultures. Sci Total Environ 439:8–17
44. Kim JH, Lee Y, Kim EJ, Gu S, Sohn EJ, Seo YS, An HJ, Chang YS (2014) Exposure of iron nanoparticles to arabidopsis thaliana enhances root elongation by triggering cell wall loosening. Environ Sci Technol 48(6):3477–3485
45. Kim J-H, Oh Y, Yoon H, Hwang I, Chang Y-S (2015) Iron nanoparticle-induced activation of plasma membrane h+-atpase promotes stomatal opening in Arabidopsis thaliana. Environ Sci Technol 49(2):1113–1119