ACS Sustainable Chemistry & Engineering

Research Article

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Nanoparticle-Sorbed Phosphate: Iron and Phosphate Bioavailability Studies with *Spinacia oleracea* and *Selenastrum capricornutum*

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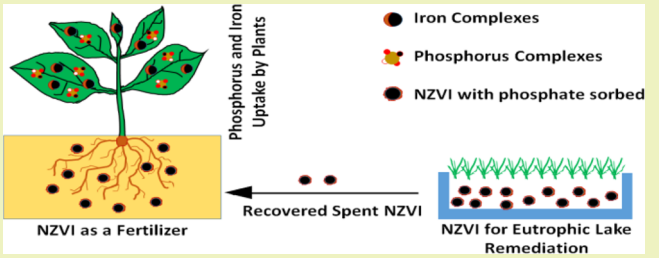
Supporting Information

ABSTRACT: In this study, nanoscale zero-valent iron (NZVI) particles have been used for phosphate recovery from aqueous solutions. The bioavailability of the phosphate sorbed onto NZVI particles was determined by using spinach (*Spinacia oleracea*) and algae (*Selenastrum capricornutum*) grown in hydroponic solutions. Simultaneous bioavailability of iron (from NZVI) was also determined. Spent NZVI particles (after phosphate adsorption) were added to the algae and spinach growth media as the only source of phosphate and iron. Phosphate sorbed by NZVI was bioavailable to both algae and spinach. The concentration of algae increased by 6.7 times when the only source of phosphate was spent NZVI as compared to algae grown in standard all nutrient media (including phosphate). Again, removing phosphate from the growth media decreased the algae concentration ~3 fold when compared to algae grown in all-nutrient media. In the spinach study, plant biomass increased in the presence of spent NZVI (where nanoparticles were the only source of phosphate) by 2.2−4 times more than the plant treated with the all-nutrient solution. Results also indicated 21, 11, and 7 times more iron content in the roots, leaves, and stems of the spinach treated with spent NZVI, respectively, as compared to the controls.



KEYWORDS: *Iron nanoparticles, Phosphate removal, Phosphate recovery, Adsorption, Spinacia oleracea, Selenastrum capricornutum, Phosphate bioavailability, Iron bioavailability, Eutrophication*

INTRODUCTION

Phosphorus (P) is a vital macronutrient for plants. Plants and other organisms mostly uptake dissolved aqueous orthophosphate and incorporate it into their tissues.[1] Phosphorus is an essential element for food production, and there is no substitute for phosphorus.[2] The amount of P in plants ranges from 0.05% to 0.30% of total dry weight.[1] Although phosphorus is abundant in most types of soils, only a tiny fraction is available for plant uptake. Low phosphorus availability for plants has been addressed by adding phosphate fertilizers to the soil. However, the amount of bioavailable phosphate is still limited due to chemical immobilization of some of the added phosphate into the soil matrix.[1] The extensive application of phosphate fertilizers leads to a phosphorus buildup in the soil, which in turn increases the potential for phosphorus loss to surface waters through surface or subsurface runoff.

Undesired loss of phosphorus and resulting nonpoint source pollution leading to eutrophication of water bodies is only one aspect of the bigger problem. The major issue with excessive use of fertilizers is the impact on global food security given the fact that phosphorus is a nonrenewable resource. Phosphorus fertilizers are produced predominantly from ores from select mines in Morocco, the western Saharan region, and China.[3] The phosphorus-bearing ore production rate is predicted to decline starting around 2035;[3] however, the use of phosphate fertilizers will be increasing under the current agriculture practices.[4] The possible short supply of phosphate fertilizers is a major concern for global food security. While there is no way to increase the amount of natural phosphorus supply, the spotlight has been shifted to sustainable practices related to phosphate fertilizers including efficient recovery and reuse of phosphates.

Almeelbi and Bezbaruah[5] have reported up to 100% removal of phosphate using nanoscale zero-valent iron (NZVI) particles and found NZVI particles to be more efficient than larger-sized particles (micro ZVI). Others have used iron oxide nanoparticles to remove (70−90%) phosphate.[6−8] Phosphate removal by NZVI and iron oxide nanoparticles is known to be a sorptive process, and the sorbed phosphate remains in the nanoparticles. It was hypothesized in this research that the sorbed phosphate (sorbed onto NZVI) would be bioavailable to plants. The objective of this research was to examine the

Special Issue: Sustainable Nanotechnology 2013

Received: February 17, 2014
Revised: May 12, 2014

   dx.doi.org/10.1021/sc500109v | ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

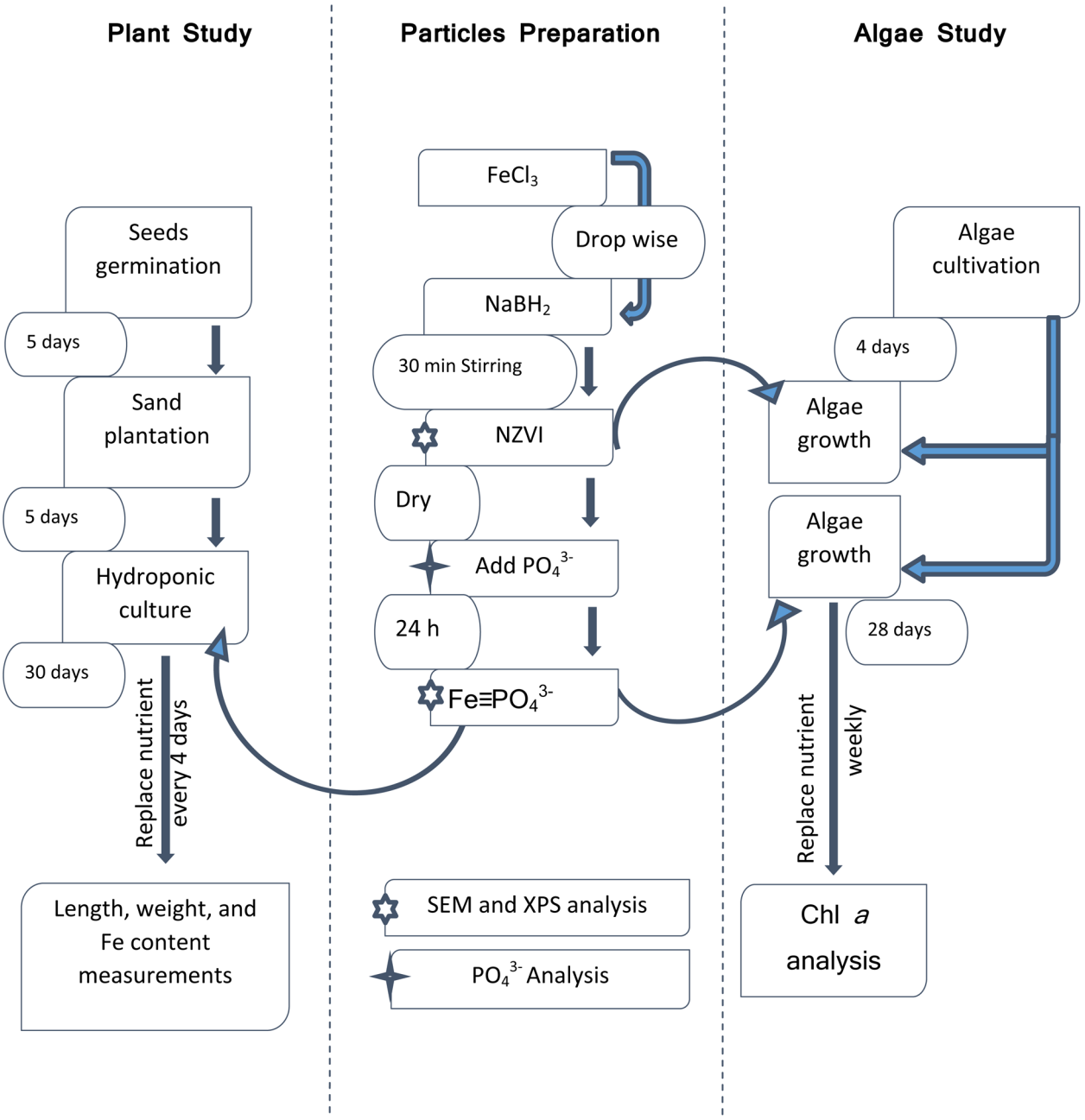


Figure 1. Schematic of the experimental design used in this study.

bioavailability of phosphate from spent NZVI (used for phosphate removal) using *Selenastrum capricornutum* and *Spinacia oleracea*.

EXPERIMENTAL SECTION

The experimental design used in this study is presented within this section (Figure 1). The particle preparation procedure and phosphate sorption experiment are discussed in brief in this paper and described elsewhere in details by Almeelbi and Bezbaruah.[5]

Chemicals. Sodium hydroxide (NaOH, BDH), calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, BDH), potassium nitrate (KNO_3, 99% pure, Alfa Aesar), potassium phosphate monobasic (KH_2PO_4, BDH), magnesium sulfate ($MgSO_4$, 97+%, Aldrich), potassium silicate-(K_2SiO_3, 99+%, Alfa Aesar), iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, Mallinckrodt), manganese sulfate ($MnSO_4{\cdot}4H_2O$, 99%, Alfa Aesar), copper(II) sulfate ($CuSO_4$, 99%, Alfa Aesar), zinc sulfate heptahydrate ($ZnSO_4$, Alfa Aesar), boric acid (H_3BO_3, Alfa Aesar), sodium molybdate dihydrate ($Na_2MoO_4{\cdot}2H_2O$, J.T. Baker), nitric acid (HNO_3, BDH), sodium nitrate ($NaNO_3$, 99+%, Fluka), calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$, BDH), potassium phosphate dibasic (K_2HPO_4, BDH), and sodium chloride (NaCl, EMD) were ACS grade and used as received unless otherwise specified.

Synthesis and Preparation of Iron Nanoparticles. *NZVI Synthesis.* NZVI particles were synthesized using the sodium borohydride reduction method (eq 1).[5]

$$2FeCl_3 + 6NaBH_4 + 18H_2O \rightarrow 2Fe^0 + 21H_2 + 6B(OH)_3 + 6NaCl \quad (1)$$

Ferric chloride hydrate (1.35 g) was dissolved in 40 mL of deoxygenated deionized (DI) water (solution A), and 0.95 g of sodium borohydride was dissolved in 10 mL of deoxygenated DI water in separate beakers (solution B). Then solution A was added dropwise to solution B under vigorous stirring conditions (using a magnetic stirrer). The resultant black precipitates (NZVI) were centrifuged and

dx.doi.org/10.1021/sc500109v | ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

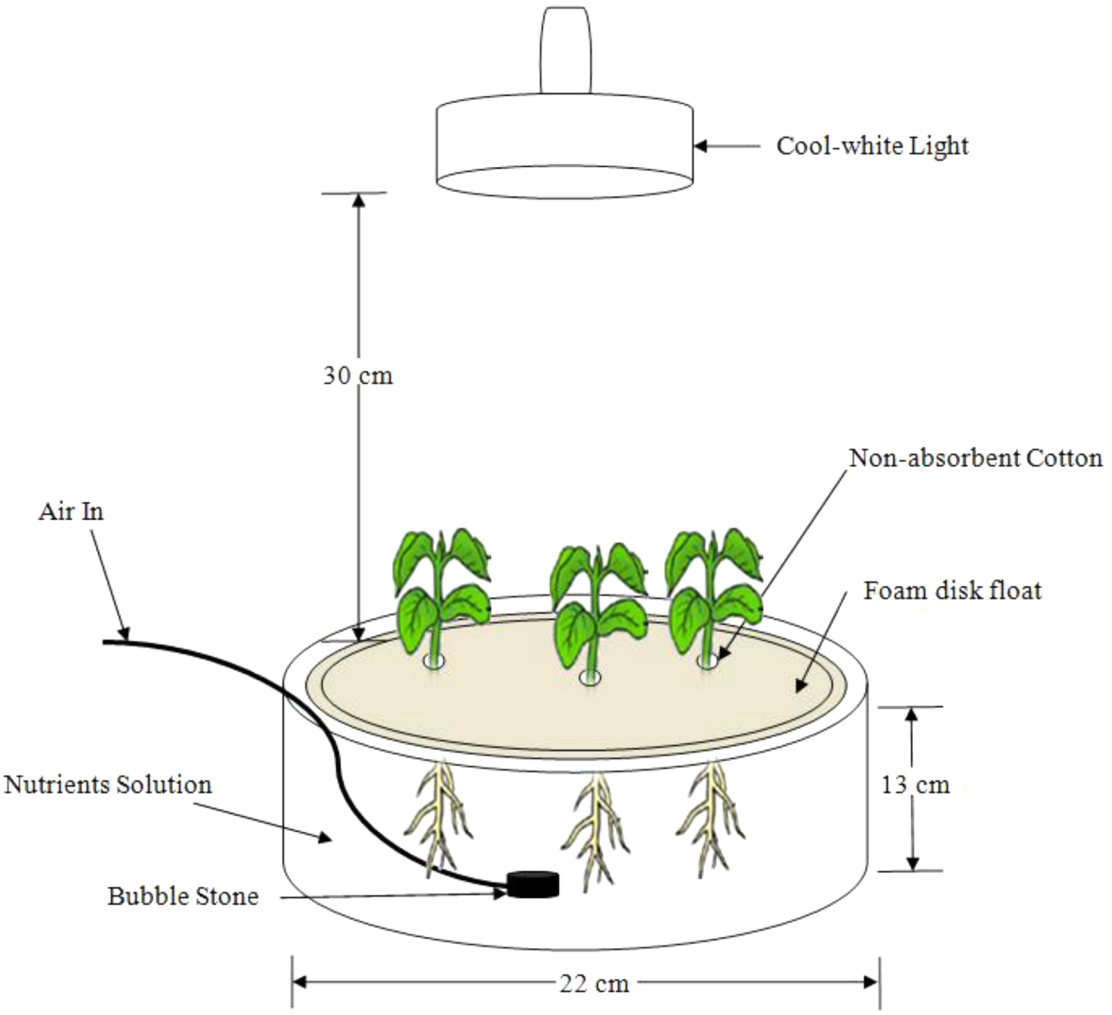


Figure 2. Schematic of hydroponic system setup used in this study.

washed with copious amounts of deoxygenated DI water and methanol to remove the undesired chemicals. The washed NZVI was dried in a vacuum oven under a nitrogen environment and then was ground using a mortar and pestle to produce NZVI particles.[5] The NZVI particles (virgin NZVI) were stored in glass vials (with head space flushed with nitrogen) for later use in experiments.

Phosphate Adsorption. NZVI (20 mg) was added toa phosphate solution (50 mL of 100 mg PO_4^{3-}-P/L) in multiple 50 mL polypropylene plastic vials (reactors) fitted plastic caps. The concentration of 100 mg of PO_4^{3-}-P/L for phosphate was decided based on adsorption capacity studies.[5] The reactors were rotated end-over-end at 28 rpm in a custom-made shaker for 24 h, and then the content was centrifuged at 4000 rpm. The supernatant was collected and analyzed for phosphate using the ascorbic acid method.[9] The precipitated iron particles were dried in a vacuum oven under a nitrogen environment and ground using a mortar and pestle. The authors have earlier reported that phosphate gets sorbed on NZVI.[5] The dried particles were characterized using X-ray photoelectron spectroscopy (XPS) and energy dispersive X-ray spectrometer (EDS) to check for the presence of phosphorus. The dried particles (spent NZVI) were used in algae and plant growth studies.

Algae Studies. *Selenastrum capricornutum* used in this study is a group of common green algae (*Chlorophyceae*) found in most fresh waters and readily available from suppliers. This species has been widely used in laboratory studies.[10−12] For algae experiments, all glassware was washed with phosphate-free detergent and rinsed thoroughly with tap water, soaked in an acid bath (10% HCl) overnight, rinsed with deionized (DI) water, and autoclaved for ∼20 min before use.

Cultivation of Algae. *S. capricornutum* (UTEX 1648) was obtained from the University of Texas Culture Collection (Austin, TX, USA).[13] An Erlenmeyer flask of 500 mL (nursery reactor) was used to culture the algal in liquid Bristol medium (Table S1, Supporting Information). The culture was aerated and illuminated with cool-white fluorescent light on a 12 h light/12 h dark cycle at room temperature (22 ± 2 °C). The light intensity was 3.17 log Lum m^{-2} (HOBO U12-012 temp/RH/light external data logger, Onset Computer Corporation, Bourne, MA, USA). The exponential growth phase was maintained as per the supplier's instructions through repetitive subculturing with freshly prepared medium every 4 days.

Growth Studies. Glass bottles (500 mL) were used as reactors, and 400 mL of different growth media and 5 mL of algae seed (*S. capricornutum*) obtained from the laboratory culture (see the Cultivation of Algae section) were added to the reactor. The algae were incubated for 28 days in the reactors illuminated with cool-white fluorescent light. During the incubation period, the reactors were manually shaken and aerated for 10 min once every day to maintain aerobic conditions. Five different growth nutrient solutions were used, and algae growth was measured at the end of the test period. Each experiment was repeated three times. The five nutrient solutions used were (i) only DI water, (ii) Bristol medium (Table S1, Supporting Information, no NZVI added), (iii) Bristol medium with virgin NZVI, (iv) Bristol medium without phosphate and no NZVI, and (v) Bristol medium without phosphate but with spent NZVI. Additional nutrients (from the stock solutions) and nanoparticles were changed once every week. Algae and spinach samples (10 mL) were collected from each reactor after 28 days, and biomass analyses were preformed immediately.

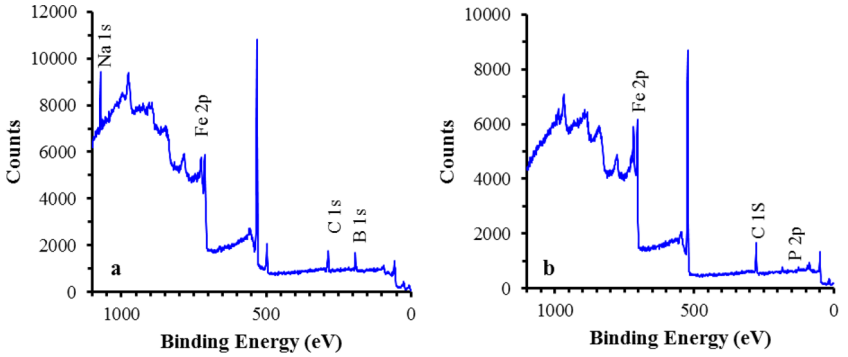


Figure 3. HR-XPS spectra of (a) virgin NZVI and (b) spent NZVI (i.e., NZVI after phosphate adsorption).

Spinach Studies. *Germination and Plant Preparation.* Spinach (Tyee spinach, *Spinacia oleracea*, Lake Valley Seed Company, Boulder, CO) seeds were purchased from a local outlet. Seeds were washed and then soaked in DI water overnight. The seeds were then placed on moist filters papers in Petri dishes and kept in the dark at room temperature until germination. The germinated seeds were planted on a sand medium in a glass tray. A nutrient solution (Table S2, Supporting Information) was added to the growth media (sand) every day, and the plants were illuminated with cool-white fluorescent light (12 h light/12 h dark cycle). The light intensity was 3.17 log Lum m^{-2}.

Growth Studies. After 5 days (during the early stage of stem and leaf formation), the spinach seedlings were removed from the sand media, and roots were thoroughly washed with DI water and transplanted into hydroponic reactors (Figure 2 and Figure S1, Supporting Information). Plastic containers (with 2 L of nutrient solution) were used for hydroponic culture. Three plants were placed into a foam disk float with the shoots supported above with nonabsorbent cotton and roots below the disk.[14] The floats with the plants were then placed in the reactors. The arrangement of putting the plants in the floats ensured continuous root contact with the nutrient solution. The nutrient solution was aerated constantly with air throughout the experiment, and the solution was replaced every 4 days. Light was provided in 14 h light/10 h dark cycles with cool-white bulbs with a light intensity of 3.17 log Lum m^{-2}. Three different treatments were run to study the effects of spent NZVI (NZVI that sorbed phosphate) on plants. In treatment 1, spent NZVI (0.15 g) was used in the reactor as the only source of phosphate for the plants. The amount of nanoparticles was decided based on the concentration of sorbed phosphate onto the particles and was equivalent to the amount of phosphate in the nutrient solution. In another container (control 1) all nutrients were used (Table S2, Supporting Information). The last treatment (control 2) had all nutrients except phosphate and iron (Fe^{3+}). Each treatment was run in triplicate. The assignments of the reactor's place in the experiment desk and plant selection (from sand media) to be put in each reactor were randomized. Each reactor was assigned a number randomly.

Analytical Procedures. *Algae Measurement.* Algae samples were collected, and the algae biomass was estimated by measuring chlorophyll *a* (Chl *a*) concentration using a pigment extraction method.[15] Ten milliliters of algal culture was filtered using Whatman GF/F glass fiber filters (pore size 0.5 to 0.7 μm, 47 mm diameter). Pigment (chlorophyll) extraction was done by soaking the filter (with algal biomass retained on them) in 5 mL of 95% ethanol and keeping it in the dark for 20 h. The solvent was then filtered through a GF/F glass fiber filter. Absorbance of the extracted sample (solvent with the pigment dissolved) was measured on a DR 5000 UV spectrophotometer using a 1 cm path length cuvette at 665 and 750 nm. The sample was then treated with 1 N HCl and absorbance was measured again at 665 and 750 nm. The following equation was used to calculate Chl *a* concentration[15]

$$\text{Chl } a \text{ (mg/m}^3 \text{ or } \mu\text{g/L)} = \frac{26.7 \times (E_{665o} - E_{665a}) \times V}{V_f \times L} \quad (2)$$

where V = volume of ethanol used for extraction (mL), V_f = water filtered (L), L = path length of cuvette (cm), E_{665o} = turbidity-corrected absorption at 665 nm before acidification ($A_{665o} - A_{750o}$), and E_{665a} = turbidity-corrected absorption at 665 nm after acidification ($A_{665a} - A_{750a}$).

To ensure reproducibility and data reliability, the experiments were ran in triplicate at different times and concentration of Chl *a* was measured in triplicate for each treatment.

Plant Measurement. Plants were harvested after 28 days of hydroponic growth. The harvested plants were washed with DI water, and the height of shoots and roots were recorded. Roots were washed with 10 mM $CaCl_2$ solution to remove NZVI physically attached onto the surface.[14] Roots, stems, and leaves were separated and then dried at 80 °C for 48 h before measuring the weight.[16] The similar parts (e.g., roots) of plants from each reactor (three plants each) were combined together, and the combined weight has been reported. Further analyses were done assuming that such combined mass as one entity.

Iron Measurement. The dry plant tissues (roots, stems, shoots) were ground and digested in a CEM Mars Xpress microwave digester. Concentrated nitric acid (HNO_3, 3 mL) was added to the ground plant tissues or standard reference material (NCS DC 73350 leaves of poplar, China National Analysis Center for Iron and Steel) in a 55 mL PFA venting vessel. Samples were divided into three groups based on their weight, and reference samples were prepared accordingly. DI water (3 mL) was added after 20 min of predigestion, and then the samples were digested at 200 °C for 15 min at 1600 W 100% power (for 28 vessels) after 10 min ramp time. The digests were analyzed for iron (Fe) and phosphorus (P) with a Spectro Genesis ICP-OES with Smart Analyzer Vision software (v. 3.013.0752) and crossflow nebulizer (three replicate measurements, 21 s integration time). Analysis of the control standard was done after every 10 samples and checked for whether it was within acceptable limits (10%).

Statistical Analysis. Analysis of variances (ANOVA) and Bonferroni Simultaneous Tests were used to analyze the data.

RESULTS AND DISCUSSION

Particles Characterization. Average particles size of virgin NZVI was found to be 16.24 ± 4.05 nm.[5] NZVI particles were analyzed using X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy with energy dispersive spectroscopy (SEM/EDS) to confirm the presence of phosphorus (P). High-resolution XPS was performed on a Surface Science SSX-100 spectrometer with an Al anode (Kα X-rays at 1486.66 eV) operated at 10 kV and 20 mA. Samples were mounted on the sample stage using conductive carbon sticky tape and transferred to the analysis chamber (with a pressure below 1 × 10^{-8} Torr). In the XPS spectrum of the virgin NZVI (Figure 3a), peaks at 711 and 725 eV represent the binding energies of $2_{p3/2}$, and $2_{p1/2}$, respectively, which can be assigned to the metallic Fe^0 and the oxide layer on the metal core. Peaks at

1071 and 192 eV BE from Na 1s and B 1s, respectively, indicate considerable concentrations of sodium (Na) and boron (B) from residual $NaBH_4$ (used for NZVI synthesis). This finding is in agreement with others.[6,17,18] The new peak at 133 eV (Figure 3b) in the spent NZVI particles is attributed to the presence of phosphorus adsorbed onto NZVI surface.[8,19]

Elemental composition of virgin and spent NZVI was determined using SEM/EDS (JEOL JSM-6300, JEOL, Ltd.). The percentage of oxygen in the virgin NZVI was found to be 12.10%. The amount of oxygen in the spent NZVI varied between 13.02 and 25.15% due to iron oxidation and phosphate sorption. Cao et al.[20] reported 8.21% oxygen in fresh (virgin) NZVI, while Krajangpan et al.[21] reported it as 15.66%. The percentage of phosphorus (total P) was found to be 7.95, 2.10, and 1.67% at three different parts in the spent NZVI (Table 1 and Figure S2b, Supporting Information). The isotherm experiments, conducted separately, determined the adsorption capacity of NZVI as 63.18 ± 7.99 mg PO_4^{3-}-P/g NZVI (i.e., 6.3%) (Figure S3, Supporting Information). The presence of a very low amount (0.51%, Table 1; Figure S2a, Supporting Information) of Na was observed in the virgin NZVI but was not present in the spent NZVI (Figure S2b, Supporting Information). Na was possibly left as the residual from sodium borohydride ($NaBH_4$) used in the NZVI synthesis process, but it is not clear why it was not detected in the used sample.

Table 1. Weight Percentage of Elements Present in Virgin and Spent NZVI Determined with EDS (SEM-EDS)[a]

		% weight			
particle type	part number	O	Fe	Na	P
virgin NZVI	1	12.10	87.39	0.51	0.00
	2	10.37	89.32	0.31	0.00
	3	10.90	88.70	0.39	0.00
spent NZVI	1	25.15	66.90	0.00	7.95
	2	13.13	84.77	0.00	2.10
	3	13.02	85.31	0.00	1.67

[a]The part numbers used for analysis are identified in the SEM images (Figure S2a,b, Supporting Information).

Algae Growth. The concentration of chlorophyll *a* (Chl *a*) is an indicator of algae health and a measure of growth. Chl *a* increased substantially when the treatments with virgin NZVI and phosphate-sorbed NZVI were used as compared to other treatments (Table 2; Figure 4). The Bonferroni test ($\alpha = 0.05$) identified two groups of experimental data based on the statistical significance. The first group included algae treated with (i) DI water (batch 1-A), (ii) all nutrients (1-B), and (iii) all nutrients except phosphate (1-C), and the algae treated with (i) virgin NZVI (2-A) and (ii) spent NZVI (2-C) particles belonged to the second group.

Table 2. Concentrations of Chlorophyll *a* at 0 and 28 Days of Algae Growth

		chlorophyll *a* concentration (μg/L)	
batch	medium	0 day	28 day
1-A	DI water	20.80 ± 1.83	81.58 ± 22.84
1-B	all nutrients	20.80 ± 1.83	300.38 ± 14.59
1-C	all nutrients (No PO_4^{3-})	20.80 ± 1.83	107.54 ± 45.73
2-A	all nutrients (No- PO_4^{3-}) + spent NZVI	20.80 ± 1.83	2002.50 ± 981.45
2-B	all nutrient + virgin NZVI	20.80 ± 1.83	1673.20 ± 270.10

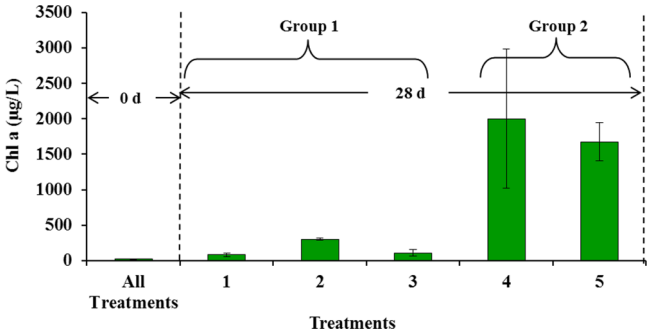


Figure 4. Chl *a* concentrations at 0 and 28 days. Treatments were as follows: (1) DI water, (2) all nutrients, (3) all nutrients (no phosphate), (4) all nutrients (no phosphate) + spent NZVI (with phosphate sorbed onto NZVI), and (5) all nutrients + virgin NZVI. The vertical error bars represent ± standard deviations. The measured values for treatments 4 and 5 were 2003 ± 981 and 1673 ± 270 μg/L, respectively.

From the first group, the algae batches treated with DI water provided the baseline data for comparison. There was a slight increase in the concentration of Chl *a* when all nutrients except phosphate were added as the growth media (from 21 to 108 μg/L, 1-C). The increase was very similar to that observed in the DI water batch (from 21 to 82 μg/L, 1-A). In the presence of all nutrients (including phosphate), the Chl *a* concentration increased from 21 to 300 μg/L (1-B), which is 2.8 times higher in growth compared to the batch without phosphate (1-C). It should be noted that all treatments (including DI water batch) had some initial growth nutrients as the seed algae was grown in Bristol media (Table S1, Supporting Information), and the some nutrients were transferred to each batch when 5 mL of seed was taken from the nursery reactor. The results from the second group showed a significant difference from the first group. The algae batch treated with all nutrients and virgin NZVI (2-B) showed an increase in algae concentration from 21 to 1673 μg/L, which is 5.6 times more growth compared to when only the nutrient solution (1-B) was used. When spent NZVI particles (with phosphate sorbed onto them acted as the phosphate source) were used, the algae growth was even more profuse and grew from 21 to 2003 μg Chl *a*/L (6.7 times higher growth than batch 1-B). It is very evident that the presence of iron nanoparticles significantly increased the growth of algae. The growth of algae was profuse when spent NZVI apparently supplied the phosphate needed for algae growth, and the final algae concentration was 6.7 times more than the batch with all nutrients (no NZVI, 1-B).

The presence of nanoparticles definitely played a major role in algae growth as is evident from the comparison of data obtained from the two groups. However, it is difficult to postulate a reason for that. The bioavailability of iron from NZVI may be a possible reason for enhanced algae growth. It is worth mentioning that the Bristol media do not contain iron as a nutrient for algal growth. Kadar et al.[22] have reported a normal growth of two different types of marine algae (*Pavlova lutheri* and *Isochrysis galbana*) in the presence of NZVI. However, *Tetraselmis suecica* showed a 30% higher growth rate in the presence of NZVI.[22] Another study[23] has indicated that the presence of iron in the growth media affected the algae growth of marine microalgae (*Chlorella vulgaris*). However, Ruangsomboon,[24] reported no significant effect of iron on

Table 3. Lengths and Weights of Plants Parts under Different Treatment Conditions

	length (cm)		weight (mg)	
treatment	roots	shoots	stem	roots
blank[a]				
control 1[b]	3.80 ± 1.04	5.89 ± 0.59	16.51 ± 4.66	3.82 ± 0.25
control 2[c]	3.22 ± 0.54	5.78 ± 0.96	6.04 ± 1.30	2.47 ± 0.65
phosphate sorbed nanoparticles	13.06 ± 2.76	20.94 ± 0.35	40.42 ± 12.35	15.27 ± 7.03

[a]DI water. [b]All nutrients. [c]All nutrients but phosphate and iron.

green algae (*Botryococcus braunii*) biomass while using $FeSO_4$ as the source of iron.

The comparison between the two batches in the second group indicates that phosphate sorbed onto NZVI was possibly bioavailable for algal growth. Phosphate plays a major role in algae growth as could be observed from the Chl *a* growth in batches 1-B and 1-C (Table 2). The final concentration of Chl *a* without phosphate (108 μg Chl *a*/L in 1-C) was 2.8 times less than the Chl *a* concentration when the nutrient solution contained phosphate (300 μg Chl *a*/L in 1-B). Others have also reported phosphate as an essential nutrient for algal growth.[25] On the basis of the algae growth observed in batches 2-A and 2-B (Table 2), it is reasonable to say that phosphate sorbed onto NZVI was bioavailable to algae.

Spinach Growth Study. Spinach seed germination started after 5 days and continued until 10 days (Figure S4, Supporting Information). The percent of seed germination varied from 72% to 100%. Plants with similar germination time and growth were transferred to the sand culture (Figure S5, Supporting Information) and later selected for the hydroponic batch studies.

Root and Shoot Lengths. *Spinacia oleracea* plants were harvested after 30 days of hydroponic growth. The length of shoots and roots were measured immediately after harvesting (Table 3 and Figure 5). In the plants treated with spent NZVI particles (with phosphate sorbed onto them), the lengths of roots and shoots were 13.06 ± 2.76 and 20.94 ± 0.35 cm, respectively. The lengths of roots and shoots in control 1 (plants treated with all nutrients as in Table S2, Supporting Information) were 3.80 ± 1.04 and 5.89 ± 0.59 cm, respectively, while the corresponding values for control 2 (all nutrient but no phosphate and iron) were 3.22 ± 0.54 and 5.78 ± 0.96 cm, respectively. The Bonferroni test (α = 0.05) put the data sets into two groups with data from spent NZVI in the first group and data from the two controls in the second group based on statistically significant differences. Plants treated with only DI water showed no growth and died within 10 days.

When the lengths of roots and shoots from the NZVI-treated plants are compared with those from control 1, it was evident that the presence of the spent NZVI had affected plant growth. The roots and shoots of the plants treated with spent NZVI were ~3.5 times longer than the roots and shoots from plants in control 1. This observation, however, does not help in concluding that phosphate and iron from NZVI were bioavailable given the fact that there are no significant differences in data obtained from control 1 and control 2. However, visual observation (Figure S6, Supporting Information) indicates that plants supplied with phosphate and iron (Figure S6a,c, Supporting Information) were healthier and the leaves were vibrant green, while control 2 (no phosphate and iron, Figure S6b, Supporting Information) had weathered leaves and the stems were skinnier. It is, therefore, safe to conclude that plants treated with spent NZVI and control 1 (all nutrients) had used phosphate.

Plant Biomass. The shoots and roots biomass of individual plants from each of the three groups of plants after 30 days was measured (Table 3; Figure 6). Plants grown in only DI water died after 10 days, and no measurement could be recorded.

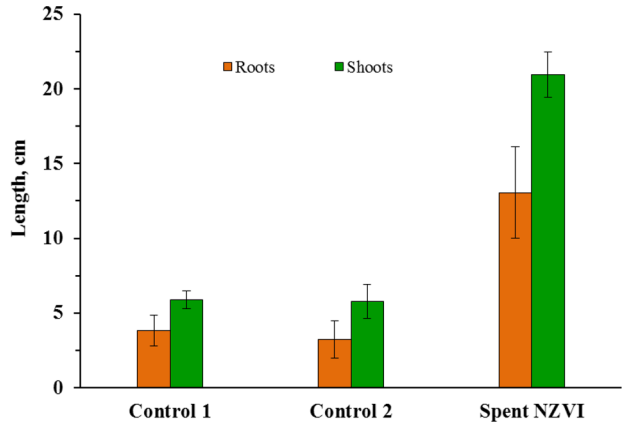


Figure 5. Lengths of roots and shoots after 30 days of hydroponic growth. Control 1: All nutrients. Blank: All nutrients but no phosphate and Fe. The vertical error bars represent ± standard deviations.

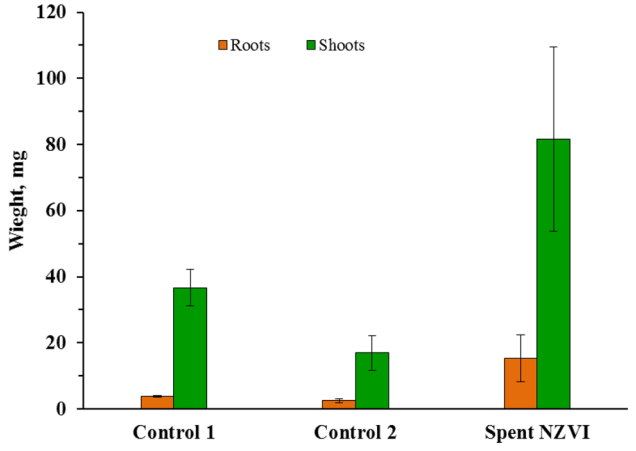


Figure 6. Weights of roots, stems, and leaves biomass. The vertical error bars represent ± standard deviations.

In the plants treated with spent NZVI particles (with phosphate sorbed onto them), the average biomass of roots and shoots (per plant) were 15.3 ± 7.0 and 81.7 ± 2.8 mg, respectively. The biomass of roots and shoots in control 1 (plants treated with all nutrients, Table S2, Supporting Information) were 3.8 ± 0.3 and 36.7 ± 5.6 mg, respectively, while the corresponding values for control 2 (all nutrients but no phosphate and iron) were 2.5 ± 0.6 and 16.9 ± 5.2 mg. The

dx.doi.org/10.1021/sc500109v | ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

Bonferroni test (α = 0.05) put the data sets into two groups with data from spent NZVI in the first group and data from the two controls in the second group based on statistically significant differences. The treatment with nanoparticles had a significant effect on plant biomass growth. The plants treated with NZVI had ~4 times more root biomass than control 1 and similarly were ~2.2 times higher than shoot biomass.

Iron and Phosphorus Analysis. Iron and phosphorus contents in the plants were analyzed and reported here as mg per unit plant. It is prudent to use this unit (mg/plant) to express the bioavailability of iron and phosphorus as plants may also increase biomass in the absence of these nutrients and then dividing the total uptake by the total biomass dilutes the concentration and does not give the actual total uptake by the plants. In the roots, the total iron uptake was ~21 times higher (Figure 7a) in the presence of spent NZVI (0.251 ± 0.011 mg/

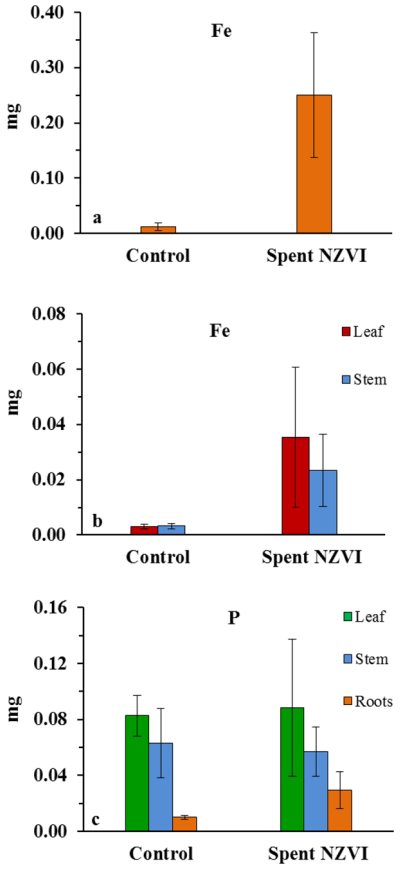


Figure 7. Iron (Fe) and total phosphorus (P) in spinach grown in all nutrients (control) and spinach grown in all nutrients (no phosphate) + spent NZVI: (a) total Fe in roots, (b) total Fe in stems and leaves, and (c) total phosphorus in stems, leaves, and roots. The concentrations were measured for each plant separately, and the average values are reported along with standard deviations. The vertical error bars represent ± standard deviations.

plant) compared to the control (0.012 ± 0.006 mg/plant). In the stems and leaves, iron uptake increased by ~7 and 11 times in the presence of NZVI (Figure 7b). The analysis of phosphorus (total P), however, did not indicate any increase in uptake because of the presence or absence of spent NZVI (Figure 7c). This may be because plants uptake only the required amount of phosphorus needed for plant growth. The fact that equal amounts of phosphorus were uptaken by plants in both reactors strongly supports that the NZVI adsorbed phosphate was bioavailable for plant uptake. The significant increase in iron concentration in the plant tissues indicates that the iron from NZVI was bioavailable as well. The bioavailability of iron from spent NZVI for plant uptake is a significant finding as iron is otherwise deficient in most human food items. Fortification of food with iron is a common practice to ensure its availability in human food.[26] While further studies will be needed to determine edibility of the bioavailable iron (transferred from nanoscale iron), it adds value to NZVI as a product for application in environmental resource (e.g., phosphate) recovery and reuse.

CONCLUSIONS

In this study, the bioavailability of phosphate and iron from phosphate-sorbed iron nanoparticles was examined using *Selenastrum capricornutum* (algae) and *Spinacia oleracea* (spinach). NZVI was synthesized and used for phosphate removal from an aqueous solution. Particle characterization using HR-XPS and SEM/EDS confirmed the presence of the phosphate on the surface of nanoparticles. Algae growth increased significantly (in the presence of the iron nanoparticles (virgin and spent NZVI)). Algae growth increased by 6.7 times when spent NZVI was the only source of phosphate compared to the algae growth in a standard all-nutrient solution. It can be concluded that the phosphate sorbed onto spent NZVI was bioavailable for algal growth. The spinach growth experiment also produced similar results where the presence of spent NZVI enhanced the growth of the plants and increased the plant biomass up to 4 times as compared to the control where phosphate was supplied from the all-nutrient hydroponic solution. The iron content significantly increased in all plant (spinach) parts (roots, stems, and leaves) when spent NZVI was added to the nutrient solution. Roots of the plants exposed to spent NZVI had the highest concentration of iron (increased ~21 times as compared to the control). Iron content also increased in the stem and leaves of the plant treated with spent NZVI by 7 and 11 times, respectively, as compared to the control. It is evident that iron and phosphate was bioavailable for the plants when the only source of phosphate and iron was the spent nanoparticles. Further research is needed to consolidate the findings and evaluate phosphate-sorbed NZVI particles as a phosphate fertilizer and iron fortifier for plants. The authors are cautiously optimistic that iron nanoparticles can eventually be used not only for nutrient recovery and reuse but also for possible nutrient fortification in plants, which would add value to iron nanoparticles.

ASSOCIATED CONTENT

Supporting Information

Additional information pertaining to plant growth, SEM-EDS analysis, and nutrient solutions used. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported with funds from the National Science Foundation (Grant CMMI-1125674). Almeelbi's tenure as a graduate student at North Dakota State University (NDSU) was supported by the Saudi Cultural Mission in the United States. Metal analysis work done by Dr. Donna Jacob from NDSU's Wetland Ecology Laboratory is gratefully acknowledged.

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