Effect of zero-valent iron nanoparticles on the phytoextraction ability of *Kochia scoparia* and its response in Pb contaminated soil

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ABSTRACT

Nanotechnology-supported phytoremediation is a new approach in remediation of toxic metal polluted soils, but very little is known about the effects of nanoparticles on plant survival and performance in Pb-contaminated soil. Seedlings of *K. scoparia* were exposed to different regimes of nanoparticles of zero-valent iron (nZVI) to investigate nZVI effects on plant growth, Pb uptake and accumulation and physiological response. Results indicated that the total Pb contents in *K. scoparia* treated with low to moderate concentrations of nZVI (100-500 mg/kg) were higher than those in control, with the highest Pb accumulation capacity of 857.18 µg per pot obtained in soil treated with 500 mg/kg nZVI. Translocation of Pb from the roots to the shoots of *K. scoparia* slightly increased with nZVI content of soil from 100 to 500 mg/kg, while Pb transfer in *K. scoparia* was suppressed at higher nZVI doses. This might be related to the biomass reduction and decrease of chlorophyll content induced by high nZVI levels. Results provide a promising method to remediate Pb-polluted soil by applying proper amounts of nZVI to enhance phytoremediation performance. Selective interaction of plants and nZVI has great application prospects in the context of soil remediation.

Keywords: *K. scoparia*, lead, phytoremediation, soil, zero-valent iron nanoparticles

1. Introduction

Soil is a major sink for heavy metals released into the environment. Heavy metals in different media e.g. in soil can find their way into human bodies and exert toxic effects on human health. Over the past decades, rapid growth of population, increasing agricultural activities, waste disposal, mining and industrialization caused extensive soil contamination throughout the world [1, 2]. Heavy metals are persistent in nature and mainly do not undergo microbial degradation, therefore might be uptaken by living organisms and accumulated in their tissues. Accumulation of non-biodegradable heavy metals e.g. Cr, Pb, Cd, Ad, Ni, and Hg in human body may pose serious health problems [3]. However, toxicity of heavy metals depends on various factors such as the dose of absorbed metal as well as the route and duration of the exposure [4]. Lead (Pb) is among the most common heavy metals found in contaminated sites [5]. Based on the Agency for Toxic Substances

and Disease Registry, lead (Pb) has been ranked 2nd among the top 10 hazardous substances. Pb mainly occurs in two different oxidation states (II and IV), but the divalent lead, Pb(II), is the most prevalent and stable form of this toxic contaminant in the natural environment [6]. In addition to humans and animals, Pb(II) is a toxic heavy metal for plants causing morphological, physiological, and biochemical dysfunctions in plant species. Lead, at certain levels, may interfere with plant establishment and growth, nutrient uptake and photosynthesis. Ascending trend of catalase and peroxidase activities in Pb-stressed plants at lower Pb concentrations in soil followed by declining trend at higher Pb levels has been reported [7]. Environmental contamination of Pb(II) is gaining growing attention worldwide because of its high levels and frequent occurrence in soil and water.

Indeed, the remediation of Pb-contaminated soils brings a techno-economical challenge for researchers and decision makers. Several physical, chemical and biological approaches have been

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employed to remove heavy metals such as lead from soil such as soil flushing, soil washing and bioremediation. Among the various remediation technologies, phytoremediation is one of the most promising, ecologically friendly, and economical remediation approaches, which can uptake heavy metals and eliminate the contaminants from soil [8]. Phytoremediation, which is also known as enhanced bioremediation, has attracted remarkable attention, especially in recent decade. Phytoremediation is a faster cleanup process compared to bioremediation. Bioremediation only uses naturally occurring or introduced microorganisms to degrade or remove contaminants in soil, whereas phytoremediation employs both plants and their associated microbes to remediate soil polluted with a wide range of contaminants such as toxic metals, organics, and radionuclides. Phytoremediation is known to be a more rapid remediation technique compared to bioremediation [9]. Introduction of plant species to a contaminated site provides an opportunity to yield several indirect contaminant attenuation mechanisms. Such mechanisms can promote the removal of toxic metals from soil. However, contaminant removal mechanisms involved in phytoremediation are complex and not limited only to the direct metabolism of contaminants by plants [10]. Certain indirect attenuation mechanisms are also involved in phytoremediation, such as the metabolism of contaminants by plant-associated microbes in the rhizosphere and/or plant-induced modifications in the contaminated environment. In terrestrial species, transport of contaminants towards the plant is dominated by the uptake of water by roots, and distribution within the plant mainly relies on xylem or phloem transport [11]. Plants can uptake contaminants like heavy metals from soil and transfer them to the harvestable parts through a sub-process called phytoextraction. This process often occurs with heavy metals, radionuclides and certain organic compounds that are resistant to plant metabolism. Phytoextraction of heavy metals was reported to be ten times more economical compared to conventional remediation techniques [12]. Selected plants for phytoextraction should have rapid growth, extended root system, and high biomass production [13]. Reduction in mobility of heavy metals in soil occurs through phytostabilization. Contaminants can be immobilized from soil or sediment onto plant root surfaces, or precipitated within the plant rhizosphere [14]. Phytoremediation of Pb using *L. perenne* in soil contaminated with Pb (up to 21 g/kg) showed accumulation of ca. 1.5 g/kg Pb in plant tissue after four months [15]. In a study by Saghi *et al.* [16], 100 to 500 mg/kg Pb were applied to soil, and it was found that growth parameters of *R. rugosum* and *S. arvensis* did not decrease significantly. In addition, higher accumulation of Pb in roots of the studied plant species was observed compared to shoots. Phytoremediation is a solar-driven remediation approach with no destructive impact on soil fertility. This technology is socially and aesthetically compelling since it can be easily incorporated into landscape design. There is also a possibility to combine phytoremediation with other techniques to enhance its effectiveness [5, 8, 11].

The use of nZVI as a feasible and prospective strategy to remediate heavy metals-polluted soils has been suggested by several researchers [16, 17]. Immobilization of heavy metals in soil through applying nZVI has been found to be a principal mechanism by which remediation goals at metal-contaminated sites can be

achieved. However, the effectiveness of nZVI to immobilize metals in soils depends on various factors, such as soil properties, dose of applied nZVI, and presence of non-target pollutants. Immobilization of anionic heavy metals (As and Cr) were greater in acidic soils, while retention of cationic heavy metals (Pb, Cd and Zn) were found to be higher in calcareous soil. Immobilization of Pb, As and Cr increased more than 82% through application of 10% of nZVI; however, nZVI could not significantly affect immobilization of Cd (only 13% to 42%), regardless of the soil pH [16, 17].

Nanotechnology-assisted phytoremediation is a novel approach for remediation of contaminated soil, sediment, and water. To date, application of nZVI in planted soils has mostly limited to plant stresses caused by these nanoparticles [18-20], and information on promoted remediation of contaminated soils under the combined effect of plants and nZVI is still scant. Enhanced growth of plants was observed at lower levels of applied nZVI, while higher rates of nZVI caused nanotoxicity [21]. Indeed, application of high levels of some nanomaterials for remediation purposes has raised concerns, due to their ambiguous fate in the environment and probable toxic effects on living organisms, particularly on plants; however, contradictory results have been reported in the literature. Inhibitory effects of above 500 mg/kg nZVI on rice seedlings was reported [22], while application of 500 mg/L nZVI promoted root growth for *A. thaliana* [23]. Enhanced activity of soil enzyme and reduced microbial toxicity was also reported in heavy metals-contaminated soil remediated with 1% nZVI [24]. Wang *et al.* [26] found suppressed uptake of Cr by Chinese cabbage and edible rape in soil amended with nZVI. To the best of our knowledge, integration of zero-valent iron nanoparticles with phytoremediation process has rarely been investigated to remove Pb from soil, which was addressed in this research.

The main aim of this study was to investigate the remediation of Pb-polluted soil through nZVI-assisted phytoremediation. We specifically studied: (i) the effect of nZVI treatments on the biomass and length of plants and their photosynthetic chlorophyll content; (ii) uptake and translocation of Pb(II) in *K. scoparia* grown in nZVI-treated soil; and (iii) impact of nZVI on accumulation capacity and remediation potential of *K. scoparia* to eliminate Pb(II). This research could illustrate the possible pros and cons of integrated application of nZVI and phytoremediation process in removal of Pb(II) from soil.

2. Material and Methods

2.1. Soil Preparation and Analysis

The soil was collected from a depth of 0-30 cm from a non-metal-contaminated area in Southern part of Tehran Province, Iran (35°32'N, 51°24'E). The collected soil samples were sieved through a 2-mm mesh to exclude the gravel and large debris, and then air-dried (22-25°C) for one week. The sieved soil was thoroughly mixed by hand before adding Pb to soil. The obtained soil was placed in plastic pots. Each pot filled with 1 kg of soil mixed with 150 mL distilled water (DW) containing dissolved salt of $Pb(NO_3)_2$, to provide desired level of Pb in soil (20 mg/kg). In the control

pots 150 mL of clean DW was added [25]. The pots were kept in a dark room (20-24°C) and incubated for eight weeks at 75% of field capacity using tap water before planting. Chemical analysis of the pots soil was carried out prior to sowing the seeds. Briefly, the soil pH was measured in suspension using a 1:2.5 (w/v) ratio of soil-water ratio. Phosphorus was determined by Olsen P extracting solution (0.5 M NaHCO₃, pH 8.5), total nitrogen by the Kjeldahl measurement (VELP Scientifica, UDK 142, Italy). Electrical conductivity (EC) was measured using a conductivity meter in a soil-water extract (1:2.5 soil: water ratio (w/v)) [26, 27]. Organic carbon (OC) content was measured according to the Walkley-Black method, in which organic carbon is oxidized using potassium dichromate [28]. The soil texture was determined using a Bouyoucos densitometer which is classified as Clay-Loam (CL). Selected characteristics of the used soil are given in Table S1. The obtained data represent the mean value of three replicates. The electrical conductivity of the used soil was 2.15 dS/m (Table S1). High values of soil electrical conductivity i.e. greater than 4 dS/m may adversely affect plant growth regardless of soil contamination type and level [29]. The total concentrations of metals in soil samples were determined through acid digestion with a mixture of 6 mL nitric acid and 2 mL chlorhydric acid in a microwave reaction system. Obtained extract was then analyzed for metals concentrations using an atomic absorption spectrophotometry (Perkin Elmer 700) according to the standard methods. All the analytical determinations were carried out in triplicate and the mean values were reported [16].

2.2. Preparation of nZVI

Nanoscale zero-valent iron (nZVI) was selected to be used in this study because of its high specific surface area and strong reducibility. It is also pretty simpler than some other nanomaterials to be prepared and the low cost makes it an attractive nanomaterial for remediation purposes. Moreover, nZVI has been reported to pose less toxicity for the plants [16, 17, 19]. Zero-valent iron nanoparticles were prepared using borohydride reduction method. FeSO₄·7H₂O (4 g) was dissolved in 200 mL of methanol/deionized water (30%:70% (v/v)). The pH was adjusted to 6.8 using 3.8 M NaOH. The NaBH₄ (1.5 g) powder was dissolved in 10 mL of de-

ionized water and added dropwise to the prepared mixture in ultrasonic shaker at 25°C for 45 min. The mixture was then stirred thoroughly at 250 rpm for another 45 min. The obtained particles were then centrifuged at 5,000 rpm for 15 min, washed at least three times with methanol, filtered, dried under vacuum condition for 6 h and pulverized. The ferrous iron was reduced to zero-valent iron based on the following equation [30]:

The prepared nZVI nanoparticles were immediately suspended in deionized water into tightly dark, stoppered bottles at room temperature. Then, the volume of the suspension was made up to 100 mL with deionized water, containing the desired concentration of the nZVI per 100 mL deionized water. The size of nZVI were determined using transmission electron microscopy (TEM), manufactured by PHILIPS (EM208 S), with an acceleration voltage of 100 kV. Selected TEM images of the synthesized nZVI are shown in Fig. 1. The size of the nZVI particles covers a range between 40 and 100 nm. Selection of nZVI concentration range was based on the preliminary experiments, which showed that nZVI concentration of lower than 100 mg/kg had negligible influence on plant growth and metal uptake. Applying higher than 1,500 mg/kg nZVI to soil exhibited severe inhibitory effects on plant growth. Therefore, the nZVI concentration range was selected as 100-1,500 mg/kg to assess the impacts of nZVI on phytoremediation of Pb-contaminated soil.

2.3. Pot Experiment

Plant seeds were obtained from the National Plant Gene Bank of Iran (NPGb) and sown in plastic pots containing different concentrations of nZVI. Nanoparticles of ZVI at doses of 0, 100, 300, 500, 1,000 and 1,500 mg/kg were added to the prepared plastic pots containing Pb-contaminated soil and thoroughly mixed. *K. scoparia* was cultivated over a 60 d period in a greenhouse. The seeds were planted in the 1.5-2.0 cm depth of the surface soil in each pot. Pots were kept in a greenhouse under natural sunlight (19-25°C, 10-12 h light) to imitate real-world conditions, and irri-

Fig. 1. TEM images of the synthesized nanoparticles of zero-valent iron.

gated two or three times per week to 75% of the field capacity of the soil. Pot experiments were carried out in three replicates. Pots were monitored to assess seedling emergence rate in different treatments and grown plants were harvested after 60 d, then the biomass and length of roots and shoots were measured. Plants were dried in an oven at 70°C for 48 h to obtain dry weight of the biomass. Phytoremediation effectiveness could be limited by various factors such as pot size and nutrient availability in the confined pots. Efficiency of phytoremediation is usually restricted by root depth in real-world conditions, but it is not the case in pot experiments, and plant root often reach the bottom of pots. However, confinement of pots restricts extension of roots in greenhouse studies. Number of seeds in each pot and their density is another limiting factor. Other factors such as soil properties and climate conditions can also affect phytoremediation effectiveness.

2.4. Determination of Metals in Plant Organs

After harvesting, plant roots and shoots were thoroughly rinsed with distilled water, dried at room temperature (22-25°C), and then oven-dried at 60°C for 12 h. The obtained samples were ground through a 200 mesh. Plant samples (0.5 g) digested in a digestion tube at a temperature of 100-230°C with 4.0 mL HNO₃ and 1.0 mL HClO₄. Extracts were filtrated by a MILEXHA 0.45 µm diameter filter to obtain a clear solution, and then analyzed for the total concentration of Pb and Fe by flame atomic absorption spectroscopy (Perkin Elmer 700) [31, 32].

2.5. Bio Concentration and Translocation Factors

The bio concentration factor (BCF) is used to calculate the metal uptake capacity from soil to plant tissues. It can be measured for each plant part, such as roots and shoots. Translocation factor (TF) is also an important tool to assess the potential of a given plant for phytoremediation purposes. It is determined from the ratio of the concentration of an element in plant's shoots compared to that in the plant's roots. BCF and TF were calculated using the following equations [33]:

$$BCF = \frac{\text{Concentration of metal in roots}}{\text{Concentration of metal in test soil}} \quad (2)$$

$$TF = \frac{\text{Concentration of metal in shoots}}{\text{Concentration of metal in roots}} \quad (3)$$

2.6. Chlorophyll Content

Fresh leaves materials were analyzed in dark condition to determine chlorophyll content in different treatments. Accurately weighted amount (0.5 g) of intact leaves were grinded thoroughly, 20 mL of 80% acetone was added. Milled mixture was then incubated at 4°C for 3 h, and centrifuged for 5 min at 2,500 rpm to remove particulate matter. The supernatant was filtered and transferred into a 50 mL volumetric flask and the volume was made up to 50 mL by addition of 80% acetone. Chlorophyll content was measured by UV spectrophotometry at 645 nm and 663 nm [34]. The chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*), and chlorophyll *a*+*b* (total chlorophyll) contents were calculated using the following equations [35]:

$$Chl\ a = 12.7 \times A663 - 2.69 \times A645 \quad (4)$$

$$Chl\ b = 22.9 \times A645 - 4.68 \times A663 \quad (5)$$

$$Chl\ (total) = 8.02 \times A663 - 20.21 \times A645 \quad (6)$$

2.7. Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 24. All results in this paper were presented as the mean with standard errors (n = 3). Significance of differences was determined using one-way analysis of variance (ANOVA), followed by least significant difference (LSD) test. Significance level was considered at P = 0.05.

3. Results and Discussion

3.1. Plant growth

3.1.1. Germination of *K. scoparia*

Seedling emergence of *K. scoparia* in different treatments was monitored during the experiment. Applying nZVI concentrations of up to 500 mg/kg to soil did not change germination rate compared to the control. However, nZVI with concentrations of 1,000 and 1,500 mg/kg significantly reduced *K. scoparia* germination (Fig. 2). Exposure of peanut seed to nZVI at low concentrations stimulated the germination and development of seeds, which was attributed to the internalization of nZVI by the plants [36]. In this study, delayed germination was also observed due to addition of 1,000 mg/kg and 1,500 mg/kg nZVI. However, *K. scoparia* showed high tolerance to the presence of elevated levels of nZVI, such that the lowest germination rate only dropped to 85 ± 5% in 1,500 mg/kg nZVI treatment. The ability to adapt harsh conditions under environmental stress is an important factor for a plant to be opted for remediation purposes.

Fig. 2. Final seedling emergence of *K. scoparia* in Pb-contaminated soil treated with nZVI. Error bars represent standard deviation of three replicates. Different letters represent significant differences between the treatments (mean ± SD; n = 3; p < 0.05).

3.1.2. Biomass of the roots and shoots of *K. scoparia*

Biomass and length of roots and shoots were determined after harvesting to assess the effects of nZVI treatment on plant growth. Fig. 3 (a) shows that applying nZVI to Pb-contaminated soil reduced root biomass of *K. scoparia* particularly at higher nZVI doses, while enhanced shoot biomass of plants in all treatments compared to the control. Decrease in roots biomass with the rising of nZVI levels was observed. The most significant increase in shoots biomass (54%) was gained in 500 mg/kg nZVI treatment, compared to the control (Fig. 3 (a)). Total plant biomass increased with the application of 100 to 500 mg/kg nZVI, while declining trend was achieved in treated soils with higher nZVI concentrations. The extent of plant growth promotion or inhibition in presence of nanoparticles in soil was suggested to be related to plant type as well as nanoparticles types and concentrations [37]. *K. scoparia* is a reasonably fast growing species that makes it suitable for phytoremediation purposes. Establishment of considerable biomass is a crucial factor affecting phytoremediation potential of plants [38]. The length of roots followed the same trend as root biomass in response to addition of nZVI to soil and declined with increasing nZVI concentrations (Fig. 3 (b)). The growth of *K. scoparia* in the test soil treated with 1,500 mg/kg nZVI with the root length of 9 ± 0.8 cm and shoot length of 13.9 ± 0.3 was significantly suppressed in comparison with the growth yielded in the control treatment. Preliminary tests showed that germination and growth of *K. scoparia* were not altered in presence of applied concentration of Pb in soil compare to the clean soil (control), and highest possible growth was achieved in the test soil. Moreover, pre-tests showed inhibitory effect of 2,000 and 3,000 mg/kg nZVI on germination and growth of *K. scoparia*, which markedly inhibit seedling emergence and plant growth. Amplified phytotoxicity of heavy metals by addition of nZVI to soil has been reported [36]. Sensibility of root extension and biomass of *K. scoparia* to high concentrations of nZVI (mainly greater than 1,000 mg/kg) was found in this study, whereas lower concentrations of applied nZVI mostly promoted plant growth in Pb-contaminated

soil. To be brief, low concentrations of nZVI favored plant growth, while elevated concentrations of nZVI i.e. 1,000 and 1,500 mg/kg in soil posed inhibitory effects on growth of *K. scoparia*. In addition, leaves of the *K. scoparia* withered more rapidly after addition of higher doses of nZVI in Pb-contaminated soil.

3.2. Phytoextraction of Pb from Soil

Phytoextraction is known to be the main mechanism by which heavy metals are remediated in soil during the phytoremediation process [39]. In this study, application of nZVI-assisted phytoremediation of Pb-contaminated soil was aimed to promote Pb uptake and translocation in *K. scoparia* or enhance immobilization of Pb in the rhizosphere of *K. scoparia*. Table 1 presents the distribution of Pb in plant roots and shoots in different treatments. Concentrations of Pb in roots of *K. scoparia* were 86.48, 112.52, 142.22, 90.42 and 60.2 mg/kg in treated soils with 100, 300, 500, 1,000, and 1,500 mg/kg nZVI, respectively, compared to 64.06 mg/kg of the control. Concentrations of Pb in roots and shoots of *K. scoparia* grown in presence of 500 mg/kg nZVI were 142.22 mg/kg and 49.78 mg/kg with a significant increase of 1.22 and 1.68 times, respectively, compared to the control. The concentrations of Pb in roots and shoot of *K. scoparia* cultivated in treated soil with 1,500 mg/kg nZVI were 60.20 mg/kg and 18.06 mg/kg, showing a slight decrease of 6.02% and 2.80%, respectively, compared with the control treatment. Pb concentrations in the roots were higher than those in the shoots in all nZVI amended and unamended treatments, indicating that *K. scoparia* roots were the preferential Pb storage organ. Reduction in concentrations of Pb in roots of *K. scoparia* under the effect of high doses of nZVI could be related to the large specific surface area of nZVI and their high adsorption capacity, which might reduce availability of Pb for being uptaken by the plant roots [40]. Besides that, inhibitory effects of high doses of nZVI due to their toxicity could reduce Pb absorption by *K. scoparia*, as also suggested by other authors [41]. Accumulation of heavy metals in plants may induce oxidative

Fig. 3. The biomass (a) and length (b) of *K. scoparia* grown in different nZVI treated soil samples at 60 d. Error bars represent standard deviation of three replicates. Different letters represent significant differences in roots and shoots, respectively, between the treatments (mean $\pm$ SD; $n = 3$; $p < 0.05$).

Table 1. Distribution of Pb in Roots and Shoots of *K. scoparia* Grown in Pb-contaminated Soil in Different nZVI Treatments, Bio Concentration factors (BCF) and Translocation Factors (TF) of Pb in *K. scoparia* Grown in Different Soil Treatments after 60 d. Standard Deviations for Three Replicates Are Presented

nZVI concentration (mg/kg)	Pb concentration (mg/kg)		Bio concentration and translocation factors	
	Roots	Shoots	BCF	TF
0	64.06 ± 1.00	18.58 ± 2.04	0.88 ± 0.02	0.29 ± 0.03
100	86.48 ± 4.72	26.81 ± 2.40	0.93 ± 0.03	0.31 ± 0.02
300	112.52 ± 5.20	37.13 ± 2.55	1.12 ± 0.08	0.33 ± 0.03
500	142.22 ± 4.47	49.78 ± 5.87	1.23 ± 0.10	0.35 ± 0.04
1,000	90.42 ± 4.63	29.84 ± 5.86	1.08 ± 0.03	0.33 ± 0.08
1,500	60.20 ± 2.29	18.06 ± 1.23	0.96 ± 0.06	0.30 ± 0.01

stress due to the production and accumulation of reactive oxygen species (ROS). Low concentrations of nZVI were found to slightly relieve the oxidative stress in plants cultivated on heavy metal-contaminated soils, while elevated oxidative stress due to addition of high concentrations of nZVI has been reported in the literature [19]. Therefore, the high accumulation of both nZVI and Pb may induce significant oxidative stress and damage to antioxidant enzymes of plants. Heavy metals such as Pb may also cause chlorosis, alter water balance and nutrient assimilation, which ultimately pose plant death. In addition, heavy metals in soil may compete with some nutrients for absorption by roots, which results in a deficiency of nutrients such as iron for plant species. Direct interaction of heavy metals with sulfhydryl group (-SH) of functional proteins is also another mechanism in heavy metal contaminated soils, which may disrupts plant functions [7, 14, 36]. Lower doses of nZVI can overcome some of these problems such as iron deficiency in heavy metal-polluted soils. However, application of higher doses of nZVI might be harmful to plant species. Nanoparticles can move along the plant pipeline upon their internalization by plants and the plant metabolism may be disturbed by excessive concentrations of nZVI [36].

The bio concentration factors (BCF) and translocation factors (TF) were calculated and presented in Table 1, to gain better understanding on the effects of nZVI-supported phytoremediation on absorption and translocation of Pb in *K. scoparia*. Increase in BCF values was observed in *K. scoparia* grown in 100-1,500 mg/kg nZVI treated soil, compared with the control soil. In the present study, BCF values were greater than one for Pb-contaminated soil amended with 300, 500, and 1,000 mg/kg nZVI. This indicates the enhanced capability of *K. scoparia* to extract Pb from the soil in presence of the mentioned concentrations of nZVI. The greatest value of BCF (1.23) was achieved by addition of 500 mg/kg nZVI, but using higher nZVI levels reduced BCF values.

Translocation of heavy metals from the roots to shoots and their accumulation in shoots is also an important index to evaluate the phytoextraction potential in plants. TF values were also slightly increased in presence of nZVI. TF values in nZVI treatments ranged from 0.3 to 0.35 for Pb, with the highest TF value obtained for 500 mg/kg nZVI treatment. The TF ratio was only slightly increased due to addition of nZVI; however, applying higher doses of nZVI showed inhibitory effects on translocation of Pb in *K. scoparia*. Higher nZVI concentrations could also adversely affect transfer

of nutrients from roots to aerial parts [36]. In addition, oxygen deficiency in soil is a possible phenomenon due to the oxidation of nZVI, particularly in treatments received high concentrations of nZVI [42]. The concentration of Pb in the roots of *K. scoparia* was higher compared to aerial parts in all treatments, indicating low ability of *K. scoparia* to transfer Pb to aerial plant organs. The limited ability of plants to transfer heavy metals to the aerial parts of plants has also been reported in the literature as a result of blocking the root apex in plants due to the exposure to heavy metals [43]. Apoplastic barriers might develop near the root apex in plants grown under the stress of heavy metals, which reduce the translocation of sorbed metals from root to aerial parts [44].

Results showed that nZVI addition to soil, particularly at lower doses, increased the uptake and translocation of Pb for *K. scoparia* in comparison with non-nZVI treatment. Stomatal opening of plant leaves could be enhanced by nZVI, leading to increased CO₂ uptake and photosynthesis [45]. Increased root length and biomass of *K. scoparia* at low doses of nZVI could help the plant to accumulate more Pb from soil as observed in this study; however, further investigation is required to gain better insight into probable mechanisms involved in this phenomenon. One probable reason is that, applying nZVI to soil may increase soil organic matter through root exudates of plants, which in turn promotes uptake and translocation of *K. scoparia*.

3.3. Accumulation Capacity of Pb

Pb accumulation capacity of *K. scoparia* does not only depend on Pb concentration in plant organs, but also depends on plant dry biomass, which was calculated and illustrated in Fig. 4. The greatest total Pb accumulation capacity in *K. scoparia* was reached 857.18 µg per pot, which was achieved in 500 mg/kg nZVI treatment. However, the least Pb accumulation capacity was calculated in 1,500 mg/kg nZVI treatment (210.80 µg per pot), followed by 1,000 mg/kg nZVI treatment (361.85 µg per pot). This indicates adverse effects of high concentrations of nZVI in soil on total Pb accumulation capacity of *K. scoparia*. Pb accumulation capacity in control treatment was slightly (4.18%) higher than that of in 1,000 mg/kg nZVI treatment. Results indicated that addition of nZVI in low doses could effectively increase the accumulation capacity of Pb for the studied plant, which is in agreement with findings of Hu *et al.* [46] who found increased As accumulation capacity of wheat in graphene oxide amended sediments. In contrast, in another study,

Fig. 4. The Pb accumulation capacity in roots and shoots of *K. scoparia* grown in different nZVI-containing soils at 60 d. Error bars represent standard deviation of three replicates. Different letters represent significant differences in roots and shoots, respectively, between the treatments (mean $\pm$ SD; $n = 3$; $p < 0.05$).

reduced upward translocation and accumulation of Cr(VI) in plant-soil system treated with biochar-stabilized nZVI was found [46]. Future application of nanomaterials in remediation works is inevitable because of their effective performance. Nanomaterials should only be used at optimal concentrations due to the fact that their excessive applications on different media such as soil may pose serious environmental hazards [47]. The environmental fate and toxicity of nanomaterials are critical issues in materials selection and design for soil remediation purposes. The knowledge about the environmental fate and toxicity of nanomaterials is still inadequate [48, 49]. Findings of this research revealed that applying proper doses of nZVI may be beneficial for plant growth with no adverse impact on plant growth parameters; while higher doses of nZVI adversely affected the plant growth. Therefore it is crucial to determine proper doses of nanomaterials for soil remediation works, as addressed in this study, to avoid excessive use of nanomaterials and prevent their associated environmental risks. In other word, for real-world applications of nanomaterials such as nZVI the first step is to implement a pilot study in order to identify

optimum conditions such as minimum required doses of nanomaterials for the effective support of phytoremediation, which might be site-specific and affected by plant species, soil type, and pollutant type and concentrations [50]. Advances in preparation of more environmental-friendly nanomaterials can also promote nanomaterials applications in the future [51]. Based on the plant growth and accumulation capacity of Pb in *K. scoparia*, it is suggested that application of low to moderate concentrations of nZVI could be promising to assist phytoremediation of Pb in soil, with the best performance at nZVI concentration of 500 mg/kg.

3.4. Fe Accumulation and Translocation

Iron is an essential micronutrient for plants growth and survival involved in cellular functions, such as DNA synthesis, nitrogen fixation and photosynthesis [52]. Concentrations of Fe in all harvested plants were determined in this research to assess the effect of nZVI-assisted phytoremediation of Pb on absorption of Fe. Table 2 indicates that the total Fe levels in roots were significantly higher than those in shoots, which may be attributed to the direct exposure of the *K. scoparia* roots to the nZVI in soil. Concentrations of Fe in roots increased with nZVI concentration to hit a plateau of 2,528.72 mg/kg in treated soil with 1,500 mg/kg nZVI, which was 2.71 times greater than the corresponding value in control treatment. In 100-500 mg/kg nZVI treatments, the concentrations of Fe in shoots as well as TF values increased with the rising of nZVI. It suggests that low contents of nZVI could promote translocation of Fe in *K. scoparia*. Despite the increase in concentrations of Fe in the roots of *K. scoparia*, translocation factors of Fe declined in 1,000-1,500 mg/kg nZVI treatments. This implied that application of high concentrations of nZVI had inhibitory effects on translocation of Fe within the plant, which might be due to the plugging of the pathway of Fe from the root to the shoot by nZVI as also suggested in the literature [22]. This signifies application of low concentrations of nZVI to successfully assist phytoremediation of heavy metals in soil because when the pathway of metals from the roots to the shoots are blocked by excessive amounts of nZVI, target heavy metals could not transfer effectively from the roots to the shoots, therefore accumulation of heavy metals in shoots could be decreased, which was in agreement with the result of Pb accumulation in *K. scoparia*. The highest total Fe accumulation capacity in *K. scoparia* was found to be 11,647.11 μ g per pot, which was also achieved in 500 mg/kg nZVI treatment. Fig. 5 illustrates

Table 2. Distribution of Fe in Roots and Shoots of *K. scoparia* Grown in Pb-Contaminated Soil in Different nZVI Treatments, Bio Concentration Factors (BCF) and Translocation Factors (TF) of Fe in *K. scoparia* Grown in Different Soil Treatments after 60 d. Standard Deviations for Three Replicates Are Presented

nZVI concentration (mg/kg)	Fe concentration (mg/kg)		Bio concentration and translocation factors of Fe	
	Roots	Shoots	BCF	TF
0	932.86 $\pm$ 24.20	326.50 $\pm$ 32.77	2.28 $\pm$ 0.07	0.35 $\pm$ 0.04
100	1366.68 $\pm$ 37.09	505.67 $\pm$ 61.97	2.43 $\pm$ 0.05	0.37 $\pm$ 0.05
300	1540.56 $\pm$ 40.07	647.04 $\pm$ 34.85	2.91 $\pm$ 0.06	0.42 $\pm$ 0.01
500	1727.51 $\pm$ 62.50	760.10 $\pm$ 81.12	3.34 $\pm$ 0.03	0.44 $\pm$ 0.06
1,000	2268.96 $\pm$ 60.75	839.52 $\pm$ 40.25	2.66 $\pm$ 0.06	0.37 $\pm$ 0.02
1,500	2528.72 $\pm$ 49.24	783.90 $\pm$ 53.19	2.64 $\pm$ 0.09	0.31 $\pm$ 0.02

that accumulation capacity of Fe in *K. scoparia* enhanced in all nZVI treatments compared to non-nZVI treatment. Table 2 indicates that the lower contents of nZVI significantly promoted Fe uptake and translocation, while higher contents of nZVI reduced uptake and translocation of Fe in *K. scoparia*. BCF and TF values of Fe in nZVI treatments ranged from 2.43 to 3.34 and 0.31 to 0.44, respectively, with the highest values obtained in 500 mg/kg nZVI treatment, which is in agreement with the corresponding tendency of Pb. Overall, accumulation and translocation of Fe by *K. scoparia* in the nZVI treated soil showed the same trend as Pb.

Fig. 5. The Fe accumulation capacity in roots and shoots of *K. scoparia* grown in different nZVI-containing soils at 60 d. Error bars represent standard deviation of three replicates. Different letters represent significant differences in roots and shoots, respectively, between the treatments (mean $\pm$ SD; $n = 3$; $p < 0.05$).

3.5. The nZVI-Induced Photosynthesis Effect

Fig. 6 shows that the accumulation of nZVI and Pb caused physiological changes in *K. scoparia*. Chl *a* and Chl *b* were used as the biomarkers of photosynthesis ability of *K. scoparia* in different treatments. Addition of low concentrations of nZVI (100-500 mg/kg) to the Pb-contaminated soil did not change Chl *a* and Chl *b* contents in this study. But Chl *a*, Chl *b* and the total chlorophyll contents significantly declined at higher concentrations of nZVI (1,000-1,500 mg/kg). Decreasing of chlorophyll content at higher levels of nZVI could be caused by adverse impact of high doses of nZVI on the biochemical factors i.e. lipid peroxidation in photosynthesis membranes [53]. The ratio of Chl *a*/Chl *b* could also be used as a useful indicator for environmental stress. Chl *a*/Chl *b* ratio was slightly changed at low concentrations of nZVI compared with the control; however, Chl *a*/Chl *b* ratio in presence of 1,500 mg/kg nZVI showed profound difference with the control (Fig. 6). Applying 1,500 mg/kg nZVI posed the greatest suppression rates for chlorophyll content of *K. scoparia* (22.58%) compared to the control, which may be attributed to the damaged chloroplast internal organization [54]. It was concluded that high concentrations of nZVI induced negative effect on plant photosynthesis, which was in agreement with the results of *K. scoparia* biomass and length at higher doses of nZVI.

Fig. 6. Changes of the chlorophyll content of *K. scoparia* grown in different nZVI-containing soils at 60 d. Error bars represent standard deviation of three replicates. Different letters represent significant differences in roots and shoots, respectively, between the treatments (mean $\pm$ SD; $n = 3$; $p < 0.05$).

4. Conclusions

This study presented a promising novel technique by the integrated use of nanotechnology and phytoremediation in the remediation of Pb contaminated sites. Application of low to moderate concentrations of nZVI (100-500 mg/kg) enhanced Pb accumulation capacity in roots and shoots of *K. scoparia*, in comparison with the control treatment. In contrast, the higher levels of nZVI exhibited inhibitory impacts on plant growth, and therefore Pb remediation effectiveness. Leaf physiological structure was also adversely affected by addition of high concentrations of nZVI to soil. A slight decline was observed in Chl *a* and Chl *b* contents of *K. scoparia* in presence of up to 500 mg/kg nZVI in soil, compared with the control, while significant negative effect of nZVI on plant photosynthesis was found at higher nZVI concentrations. Significantly higher accumulation of Pb in roots of *K. scoparia* compared to the shoots in all treatments suggested that phytostabilization could be the dominant mechanism in phytoremediation of Pb in both unamended and nZVI-amended soil. Low concentrations of nZVI effectively increased accumulation capacity of *K. scoparia* for Pb with plant growth promotion. Iron uptake by *K. scoparia* and its accumulation in the plant tissues exhibited similar trend as lead. Organo-metallic interactions as well as redox reactions may also play an important role in nanomaterials-assisted-phytoremediation which can be further investigated in future studies. Soil pH can also play a key role in the phytoavailability of heavy metals in presence of nanomaterials in soil, which is suggested for future researches. Engineering use of nanomaterials coupled with biological methods require significant cautions to be taken into account. In summary, the addition of low concentrations of nZVI could promisingly support *K. scoparia* growth and promote the uptake and translocation of Pb in plant. Stabilization of Pb in soil was also boosted by using

low concentrations of nZVI, which demonstrated that combined use of nZVI and phytoremediation could be a promising approach to remediate Pb-contaminated soils.

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Author Contributions

A.D.Z. (Assistant Professor) developed the theory and idea, carried out the experiments, performed and verified the analytic calculations and numerical simulations, wrote the manuscript.

A.M.T. (Associate Professor) developed the theory and idea, carried out parts of the experiments, verified the analytic calculations, wrote the manuscript.

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