

Article

Impact of Nanoscale Zero-Valent Iron on the Growth and Iron Nutrition of *Hordeum vulgare* L. and *Triticum aestivum* L. Cultivated in Alkaline Soil

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Abstract

Nanoscale zerovalent iron (nZVI) has shown promise for soil remediation. This study examined the impact of this nanomaterial on barley and wheat grown in alkaline soil throughout a complete growth cycle, to assess its potential as a fertilizer and to understand its behavior in an uncontaminated matrix. A greenhouse experiment was conducted in which barley and wheat plants were grown in soil treated with a commercial nZVI slurry at 0 and 5% (equivalent to 0 and 8 g kg⁻¹). Physiological parameters were monitored throughout the growth cycle, and plants were harvested after five months. Biomass production, impact on root and leaf ultrastructure, and the concentrations of Fe and other nutrients were determined in several plant tissues. Soil physicochemical properties were not adversely affected by nZVI application, and an increase in Fe availability was observed regardless of the species, from 1 mg kg⁻¹ to 5.2 and 6.6 mg kg⁻¹ in barley and wheat soils, respectively. However, this increase did not translate into higher Fe accumulation in plant tissues at the end of the growth cycle, nor did it enhance plant growth in either species. Therefore, under the experimental conditions evaluated, the application of nZVI as an iron fertilizer cannot be recommended. Notably, both crops exhibited a greater sensitivity to nZVI during early stages of development, as evidenced by significant reductions in chlorophyll content and increased oxidative stress. These initial adverse effects were progressively alleviated as plant growth advanced, with no detectable alterations in cellular ultrastructure, allowing both species to complete their growth cycle.

Keywords: nZVI; barley; wheat; cereal; nanofertilizer; greenhouse



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1. Introduction

The European Commission has adopted a formal definition for nanomaterials. According to the European Union Observatory for Nanomaterials (EUON), a nanomaterial is “a natural, incidental, or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm” [1]. Their minute size results in a high specific surface area and reactivity, which together confer a wide range of physical and chemical properties and functionalities. In this context, nanotechnology has experienced significant advancements in the last decade, finding applications spanning multiple fields of life, including medicine, electronics, the

textile industry, cosmetics, agriculture, and environmental science [2–6]. However, the continuous release of nanomaterials requires thorough assessment of their potential impact on the environment and human health [7].

In this regard, nanoscale zero-valent iron (nZVI) has emerged as a promising material for environmental remediation, specifically for the treatment of water and soils contaminated with metal(loid)s and/or organic contaminants [8–16]. Numerous studies have concluded that the application of nZVI to contaminated soils significantly reduces the mobility and bioavailability of metal(loid)s, thereby decreasing their toxicity and enhancing plant growth [10,17–20]. A recent study has demonstrated that nZVI application to an As-contaminated paddy soil improved rice grain yield, increased Fe absorption, and decreased As uptake [21]. Others have reported that rice plants grown in Cd-contaminated soil treated with nZVI show a significant increase in biomass and reduced Cd content [20,22]. Similar growth-stimulating effects have been observed in barley, sunflower, and ryegrass grown in soils contaminated with As, Cr, or Zn, where nZVI application effectively reduced pollutant accumulation in plant tissues [10,17,19]. However, the increasing use of nZVI for remediation also implies its potential introduction into natural environments and therefore new emerging risks [7]. Moreover, the presence of contaminants may mask or modulate the intrinsic effects of nZVI on soil properties and living organisms [23]. Therefore, studies under environmentally realistic conditions, at mesocosm and field scale, evaluating the impact of nZVI on uncontaminated soils are essential to better understand the baseline effects of this nanomaterial on soil quality and plant development, as well as to ensure its safe and sustainable use in remediation technologies [7].

In addition, the Fe present in this type of nanomaterial may become bioavailable for plants and soils. Iron is an essential element for plants and other organisms, mainly due to its capacity to participate in electron transport through changes in valence [24]. Specifically, in plants, this metal participates in multiple cellular processes, including respiration, photosynthesis, and DNA and chlorophyll biosynthesis, and it is a component of many proteins and enzymes [25]. Although Fe is one of the most abundant elements in the Earth's crust, it often shows low availability in soils because it is present in its insoluble form, mainly as $\text{Fe}(\text{OH})_3$, at neutral to alkaline pH values, as well as under aerobic conditions. Such depletion induces iron-deficiency chlorosis in plants, leading to reduced crop yields and a diminished nutritional quality [26,27]. Excess Fe can lead to cell damage and can interfere with metabolic processes, resulting in plant growth defects and decreased crop yield [28]. Furthermore, Fe deficiency in crops represents a significant public health concern, contributing to conditions such as anemia [28,29], and remains one of the most prevalent micronutrient disorders worldwide [30]. However, soil amendment with this metal is challenging, as Fe fertilizers are often expensive, environmentally harmful, and inefficient [31].

Several studies have demonstrated that nZVI particles exhibit strong adsorption onto soil components (e.g., minerals and colloids), leading to aggregation, reduced mobility, and decreased bioavailability [32,33]. Zhang et al. [33] observed that three types of natural soils strongly adsorbed nZVI particles with relatively fast kinetics. Additionally, interactions of nZVI with soil organic matter significantly influence its behavior and efficiency [32,34]. Furthermore, oxidation and aggregation can reduce nZVI reactivity [33,35]. Zero-valent iron nanoparticles tend to aggregate due to magnetic and van der Waals forces. To mitigate these limitations, various stabilizing coatings, such as surfactants, polymers, and solid supports, have been employed [35].

Zero valent iron nanoparticles have the capacity to stimulate plant growth and increase Fe content in plant tissue [29]. Studies focused on germination and early seedling development have shown that the impact of nZVI strongly depends on both the dose applied

and the plant species involved [36,37]. While high concentrations can be deleterious for plant growth, several species—including *Lepidium sativum* L., *Sinapis alba* L., and *Sorghum saccharatum* L.—have shown no adverse effects when exposed to nZVI under hydroponic conditions [38]. In contrast, the treatment of mung bean seeds with 12.5 g L^{-1} of nZVI caused severe inhibition of germination and seedling growth, along with pronounced phytotoxic stress [39]. A hydroponic study with *Cannabis sativa* L. concluded that nZVI did not outperform Fe-EDTA with respect to Fe uptake efficiency, translocation, or photosynthetic activity [40]. Most experiments on nZVI behavior have been conducted in aqueous medium over short periods. Therefore, the results from these studies cannot be directly extrapolated to soil since the bioavailability of the nanoparticles is often reduced due to their interaction with soil components. Under hydroponic conditions, dissolved elements are more readily available for plant uptake, whereas in soil they may become partially immobilized by soil particles. In addition, the effects of nZVI may vary among plant species; however, few systematic studies have addressed the long-term impact of this nanomaterial across different plant species. Moreover, soil properties play a key role in determining the effects of nZVI on plants [41,42], along with factors such as the type of nZVI used [43,44], the dose applied, and the aging of the material [43,45,46]. For instance, nZVI doses above 0.5 g kg^{-1} have been reported to inhibit rice seedling growth, although germination is not impaired [45]. In contrast, soil treated with 0.5 g kg^{-1} nZVI enhanced the growth of *Arabidopsis thaliana* L. and increased Fe uptake in roots and leaves [47]. Moreover [48] found that the physiological responses of maize exposed to nZVI over four weeks were concentration-dependent: 0.05 g kg^{-1} stimulated root elongation, whereas 0.5 g kg^{-1} increased leaf pigment content and decreased nutrient accumulation.

Overall, the literature is predominantly based on short-term studies, frequently using hydroponic systems and focusing on early developmental stages. Although nZVI has been shown to exert both beneficial and detrimental effects on plants, there is limited understanding of plant responses over the long term, especially in adult plants, whose reactions may differ substantially from those in early growth stages. This knowledge gap is particularly relevant for cereals, including wheat and barley, which are vital for global human and animal nutrition [49]. Thus, to assess the potential of nZVI as a fertilizer and understand its behavior in the context of an uncontaminated matrix, here we studied the impact of nZVI on barley and wheat plants grown in alkaline soil throughout their five-month growth cycle. Additionally, we addressed the effects of nZVI on soil properties.

2. Results and Discussion

2.1. Impact on Soil Properties

Table 1 shows the mean and standard deviation of soil physicochemical properties, including Fe availability in soil collected after harvest. Under the experimental conditions tested, nZVI did not induce major adverse changes in soil physicochemical properties after five months. The most notable effect was observed on exchangeable Na and DTPA-Fe. Soil pH was not affected by the nanomaterial despite its alkaline nature. This observation could be explained by the strong buffering capacity of the soil associated with its high initial pH. In contrast, previous experiments conducted on acidic soils, with lower buffering capacity, showed significant increases in soil pH after nZVI application [41,50,51]. Soils in the NP treatments showed a significant increase in exchangeable Na, namely 49% and 73% for wheat and barley, respectively. This effect is likely associated with the sodium polyacrylate stabilizer contained in the commercial nZVI formulation (see Section 3.2). However, the values remained within the normal range for soils and did not result in a significant increase in electrical conductivity. Iron availability in soil, measured as Fe in the DTPA extract, significantly increased with the NP treatments, regardless of plant

species. Specifically, DTPA-Fe rose from slightly above 1 mg kg⁻¹ to 5.2 and 6.6 mg kg⁻¹ in the barley and wheat soils, respectively. However, these values can be considered moderate from a soil fertility perspective. Indeed, Zhou et al. [52] reported that soils with DTPA-Fe concentrations below 6 mg/kg can be considered Fe-deficient for wheat. Iron shows low solubility under neutral and alkaline pH conditions, whereas reducing and acidic conditions promote its mobilization [53]. In this regard, higher DTPA-Fe content was observed in an acidic soil treated with 110 mL of the commercial nZVI slurry on 90 g of soil [54]. Furthermore, Fe availability may decrease over time, especially under alkaline conditions due to precipitation as Fe carbonates and hydroxides [53]. In this context, a study involving a one-month experiment with lettuce grown in an alkaline soil treated with nZVI—even at a lower dose—reported higher Fe concentrations in the DTPA extract than those found in the present study [41], in which soil samples were collected five months after nZVI application. On this point, the DTPA-Fe content in soil treated with aged nZVI (2- or 4-week-old) was found to be significantly lower than that of the treatment with fresh nZVI [45]. Another study described a decrease in DTPA-Fe in two soils (pH 7.7 and 9.3) treated with various Fe-rich materials over 30–60 days [55]. Similarly, a 32-month field-scale experiment with two polluted soils with a neutral pH showed an initial increase in TCLP-Fe immediately after nZVI addition, followed by a decline over time [13]. Moreover, no significant increase in Fe was detected at any sampling time (from one week to one year) in the porewater of a polluted soil with near-neutral pH that was treated with various Fe-based materials under field conditions [56]. Similarly, other authors reported no increase in DTPA-extractable Fe 14 days after the application of nZVI at 1 g kg⁻¹ to a near-neutral soil [57]. Of note, the use of different methodologies to evaluate Fe availability hinders the comparison of results.

Table 1. Physicochemical soil properties after plant harvest (mean ± standard deviation, *n* = 4).

Parameter	Wheat		Barley	
	C	NP	C	NP
pH	8.00 ± 0.19	8.10 ± 0.03	8.18 ± 0.16	8.06 ± 0.05
EC (dS m ⁻¹)	0.54 ± 0.15	0.36 ± 0.08	0.41 ± 0.10	0.35 ± 0.05
OM (%)	1.45 ± 0.14	1.50 ± 0.11	1.32 ± 0.16	1.44 ± 0.13
N (%)	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.00	0.11 ± 0.01
P (mg kg ⁻¹)	80 ± 12	62 ± 12	70 ± 5.2	62 ± 6.0
Ca (mg kg ⁻¹)	2979 ± 139	3100 ± 120	2685 ± 416	3117 ± 265
Mg (mg kg ⁻¹)	348 ± 28	356 ± 12	390 ± 25	378 ± 14
Na (mg kg ⁻¹)	64 ± 7.4	96 ± 19 *	65 ± 23	112 ± 28 *
K (mg kg ⁻¹)	508 ± 57	451 ± 26	346 ± 38	374 ± 17
Fe-DTPA (mg kg ⁻¹)	1.10 ± 0.08	6.55 ± 1.89 **	1.38 ± 0.05	5.23 ± 2.34 **

EC: electrical conductivity; OM: organic matter. Asterisks mean significant differences based on Student's *t*-test:

* *p* < 0.05; ** *p* < 0.01.

2.2. Impact on Barley and Wheat Plants

2.2.1. Physiological Parameters

Plants in the C and NP treatments completed their growth cycles, although the latter showed a slight delay in the first stages compared with the former. Figure 1 shows the measurements of chlorophyll content, RWC, MDA, and biomass. Chlorophyll content in the leaves of barley and wheat plants showed a significant reduction in the NP treatments only in the first sampling point (12% and 35% for wheat and barley, respectively), after which comparable values were obtained. At this sampling time, the RWC of leaves was similar for plants grown in C and NP treatments, for both species. At the second sampling point, the RWC of wheat from the NP treatment showed a significant decrease compared with control plants, but no differences were observed for barley. Thus, the water influx in

barley was unaffected by the application of nZVI under the experimental conditions tested, whereas wheat showed a reduction in this parameter at Zadoks stage 40. Previous studies have reported that excess nZVI can induce the formation of an iron plaque surrounding the roots, which may limit water uptake [11,37,42,58]. Iron plaque formation has been described in rice plants grown in the presence of nZVI; however, limited information is available for cereals such as barley and wheat, highlighting the need for further research.

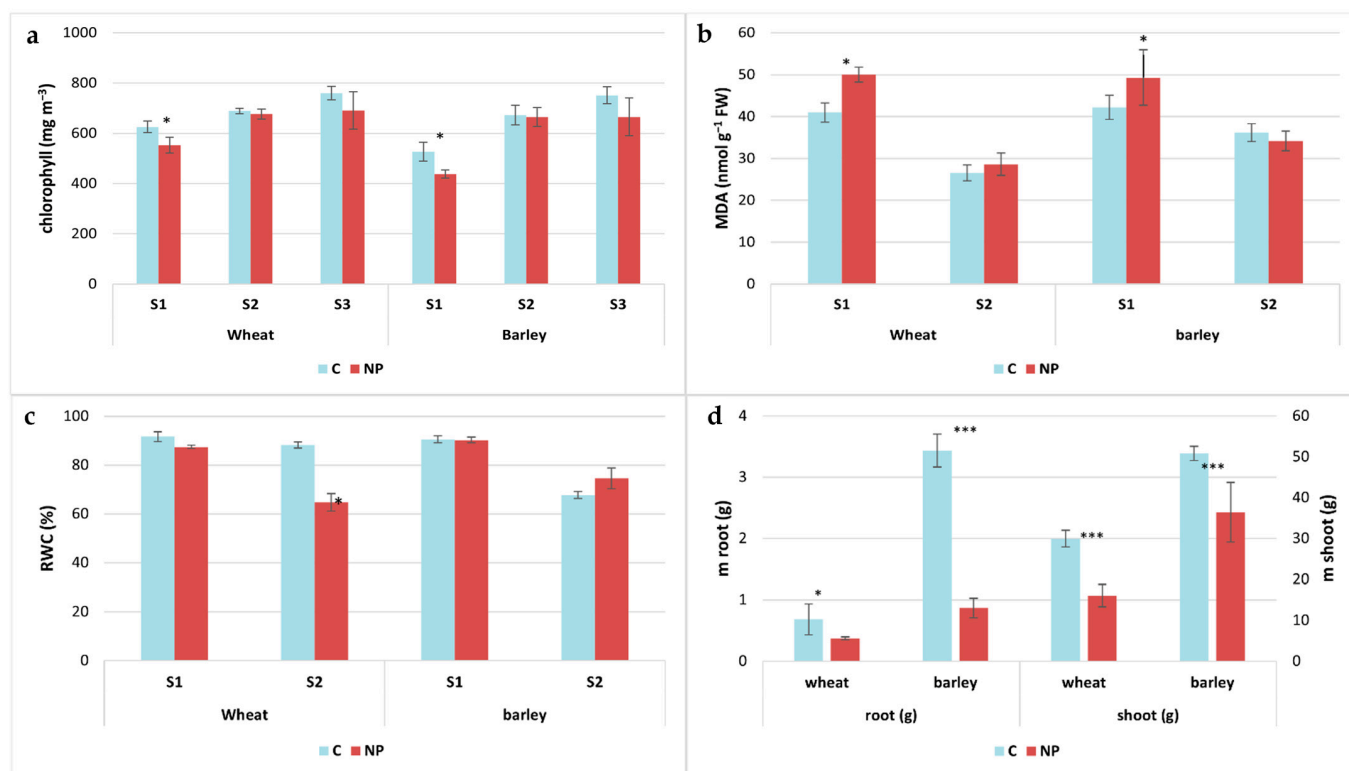


Figure 1. Total chlorophyll content, mg m^{-3} (a), MDA (malondialdehyde, nmol g^{-1}) (b), RWC (relative water content, %) (c), and biomass, g (d) of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) plants at the different treatments. The data presented are the mean concentrations $\pm$ standard deviations ($n = 4$). Error bars indicate standard deviation. Asterisks mean significant differences based on Student's *t*-test: * $p < 0.05$; *** $p < 0.001$.

Oxidative stress was evaluated by quantifying MDA. The presence of nZVI induced MDA production in both species at the first sampling point, with this parameter increasing by 22% in wheat and 17% in barley. However, at the second sampling time, both species, whether grown in C or NP treatments, showed a similar content of MDA, which was lower than at the first sampling. MDA is a biomarker of cell membrane integrity, as it is released during the decomposition of phospholipids. Excess Fe and/or redox imbalance can promote ROS production via the catalytic action of the Fenton reaction [54]. Figure S1 shows the appearance of the cereal plants three months after they were transplanted (at Zadoks stages 26 and 33, for barley and wheat, respectively). Visual evidence of toxicity was not detected in any case. In the same way, leaf chlorosis was not observed for wheat or barley throughout the experiment. However, plants of both species grown in nZVI-amended soil had significantly lower biomass than the control plants. Specifically, barley exhibited reductions of 75% and 28% in root and shoot biomass, respectively, whereas wheat showed decreases of 45% and 46%. These differential responses suggest a species-dependent sensitivity to nZVI. In this regard, a previous study comparing the impact of Cr pollution on the development of barley and wheat plants observed a similar reduction in root and shoot biomass of wheat, whereas for barley, the effect on root was notably higher

than on shoot [59]. These results suggest that, under the experimental conditions tested, the treatment did not exert a growth-promoting effect but rather inhibited plant development. However, the impact of nZVI is influenced by several factors, including plant species, soil properties, nZVI dose, and nZVI type. The application of similar or even higher doses of nZVI suspensions to acidic soils has been reported to either stimulate growth or have no significant effect on *Lolium westerwoldicum* “Breakw” after six weeks of cultivation [44]. Likewise, a study evaluating lettuce growth in acidic and alkaline soils treated with a similar nZVI dose to that used in the present study found increased chlorophyll content under both soil conditions; however, enhanced biomass production was observed only in acidic soil. Therefore, further studies under a wider range of experimental conditions are required to better understand the factors governing barley and wheat responses to nZVI and to optimize their growth [41].

Similarly, the fresh weight and photosynthetic activity of maize plants grown for four weeks in an acidic soil treated with nZVI at lower doses than those used in the present study (0.05 and 0.5 g kg^{-1}) were unaffected [48]. The type of nZVI applied is also a critical factor, as previous studies have reported higher phytotoxicity for stabilized nanoparticles compared with bare nZVI [43]. Specifically, those authors found a dose-dependent inhibition of photosynthetic activity and growth of *Vigna radiata* L. when nZVI was stabilized with starch or activated carbon. In contrast, barley plants grown in contaminated soils and treated with the same commercial nZVI suspension as that used in the present study, even at higher doses, exhibited significantly enhanced growth compared to plants grown in untreated soil [10,17]. In particular, a greenhouse experiment involving barley cultivated in Cr-contaminated soils (73 and 230 mg kg^{-1}) showed that the application of nZVI at the same dose as that used in the present study enabled plants to complete their growth cycle, whereas untreated plants died [17]. These findings suggest that the presence of soil contaminants may mask or override the direct effects of nZVI on plant growth. Further studies under different conditions are needed to better understand and promote barley and wheat growth.

2.2.2. Plant Root and Stem Ultrastructure

The impact of soil treatment with nZVI on root and leaf cell ultrastructure was evaluated by TEM (Figure 2). Overall, no obvious ultrastructural abnormalities were observed in the examined tissues. Specifically, the root cells of both plants in the C and NP treatments were highly vacuolated, typical of mature root cells, and mitochondria appeared normal and well-structured (Figure 2a–d,g–j). In leaves, chloroplasts showed abundant and dense thylakoids with well-defined grana stacks (Figure 2e,f,k,l). Thus, at Zadoks stage 70 (four months after transplanting), TEM analysis did not reveal significant differences in the ultrastructure among plants in the C and NP treatments. These results support the hypothesis that barley and wheat plants are more sensitive to the presence of nZVI during the early stages of their growth cycle, as reflected by delayed development, reduced chlorophyll content, and increased oxidative stress, ultimately leading to decreased biomass production. In contrast, the impact of the nanomaterial diminished at later stages, suggesting a degree of plant adaptation. This attenuation may be associated with the increased tolerance of mature plants and with physicochemical changes in nZVI particles, such as aggregation and oxidation to $\text{Fe}(\text{OH})_3$, which reduces their bioavailability and reactivity [45,60,61]. In this regard, the phytotoxicity of nZVI aged for 4 weeks was found to be significantly lower than that of fresh nZVI [45]. Most available literature has assessed the ultrastructure of plants exposed to nZVI over short periods, and little information is available on long-term exposure. In a previous study with lettuce plants grown in an alkaline soil treated with nZVI, TEM analysis was performed 23 days after application of the nanomaterial, finding

that the root cells of plants exposed to nZVI showed lower integrity than those of the controls [41]. A three-day hydroponic experiment performed with mung beans treated with nZVI at 12.5 g L^{-1} showed strong phytotoxicity, even causing plant mortality, together with pronounced ultrastructural alterations [39]. However, it is important to note that results from hydroponic conditions are not directly comparable to those from soil systems. In aqueous media, nZVI bioavailability is typically higher, whereas in soil, nanoparticles may interact with soil components and undergo aggregation. Additionally, in the experiment with mung beans, the acidity of the hydroponic medium can favor Fe solubility, whereas in the present study, the alkaline soil pH would limit this parameter.

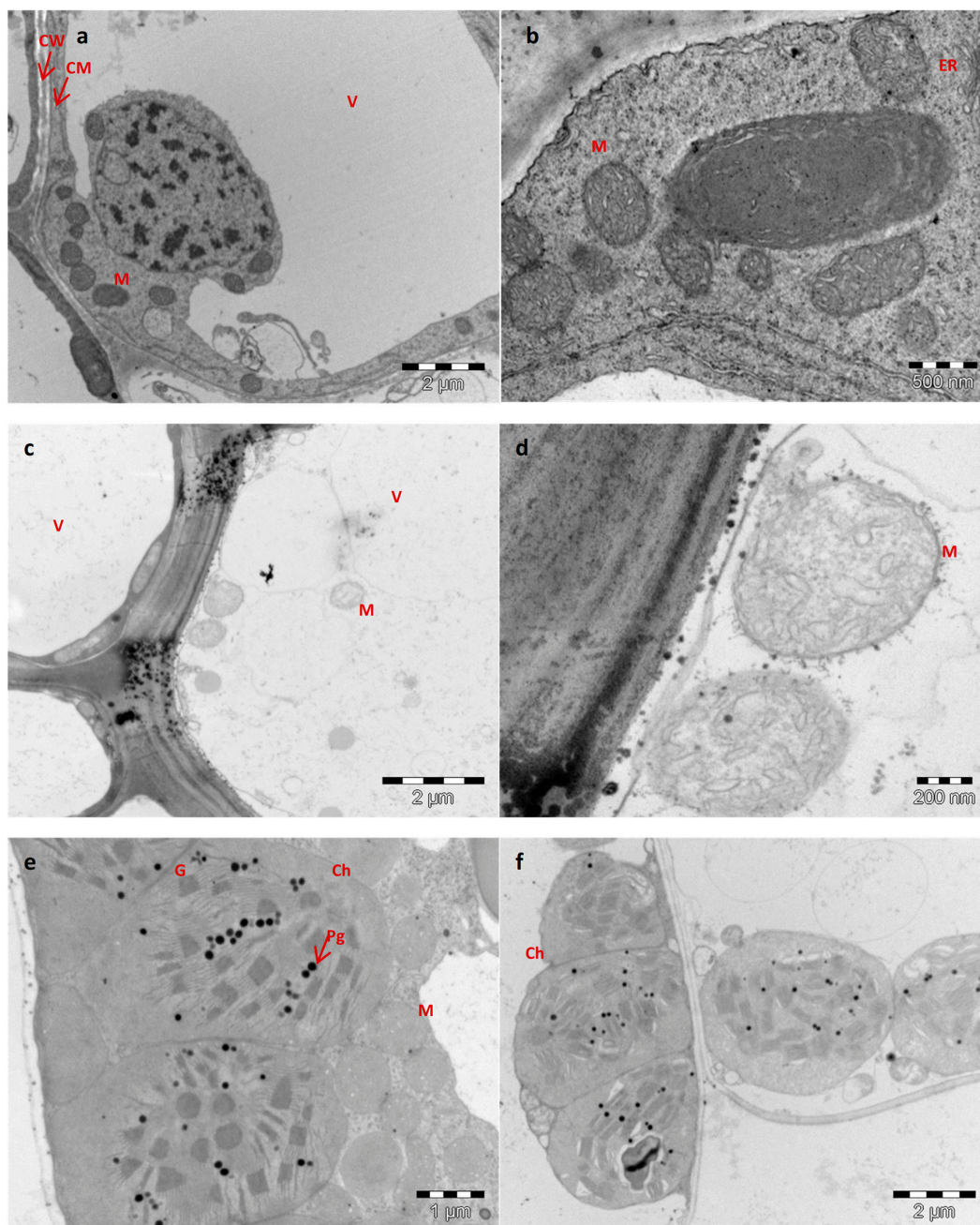


Figure 2. Cont.

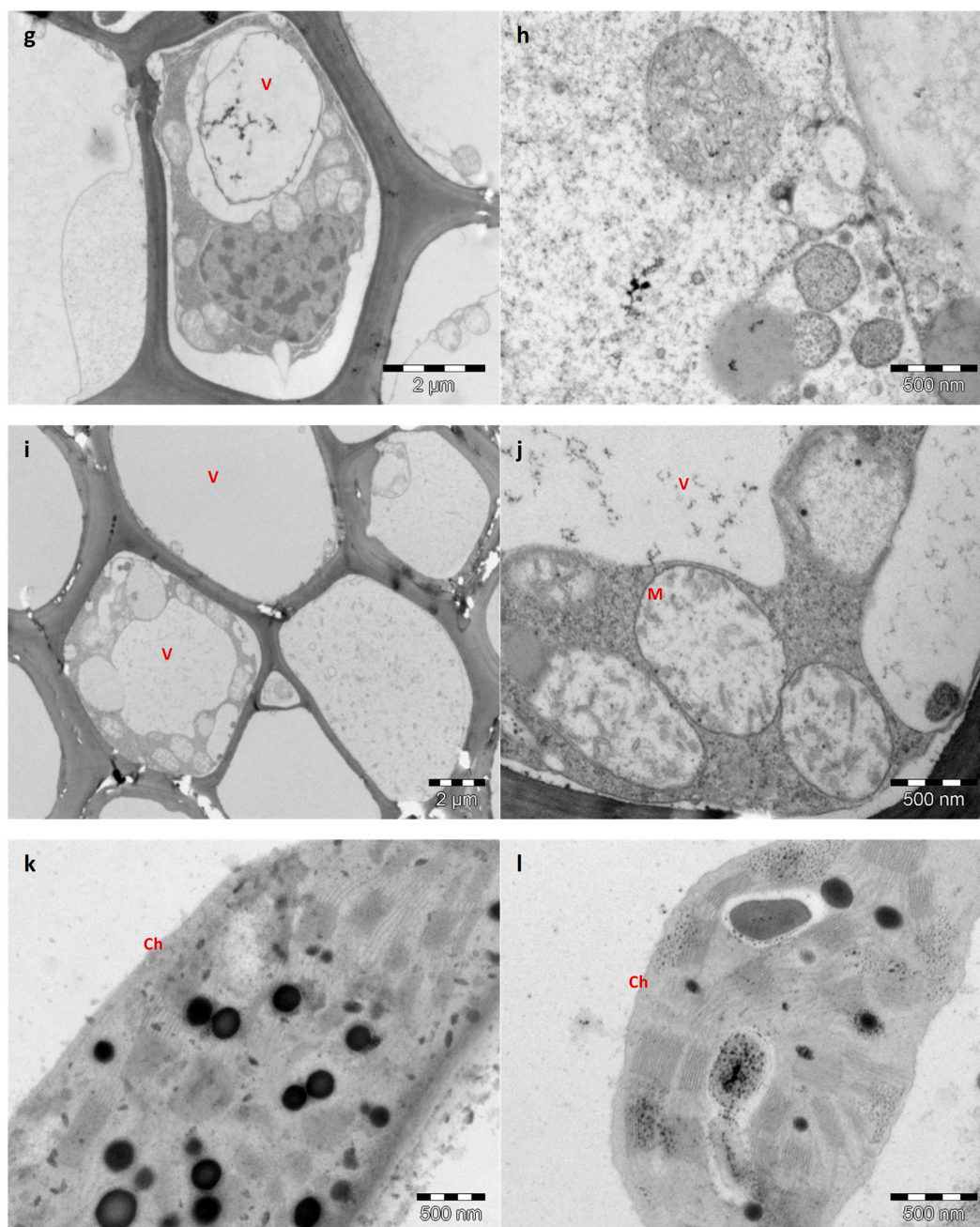


Figure 2. Transmission electron microscopy (TEM) images of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) tissue: wheat root cells from the control (a,b) and the NP treatment (c,d); wheat leaf cells from the control (e) and the NP treatment (f); barley root cells from the control (g,h) and the NP treatment (i,j); barley leaf cells from the control (k) and the NP treatment (l). Abbreviations: Ch: chloroplasts; CM: cell membrane; CW: cell wall; ER: endoplasmic reticulum; G: grana stack; M: mitochondria; Pg: plastoglobuli; V: vacuole.

2.2.3. Accumulation of Iron and Other Nutrients

Regarding Fe accumulation in plant tissues, the addition of nZVI to soil under the experimental conditions tested did not induce a significant increase in Fe concentration in barley or wheat, in either roots, shoots, leaves, or grains (Figure 3). Similar Fe contents were observed in plants from the C and NP treatments. Although the available Fe in soil increased significantly after nZVI application (Table 1), this increase did not translate into higher Fe concentrations in plant tissues at the end of the growth cycle. These results could be explained by the following factors: (i) the increase in available Fe may not have been

sufficient to enhance Fe accumulation in plant tissues; and (ii) Fe uptake rates vary over time, depending on nZVI aging and plant physiology [30,45]. In this context, a previous study on rice plants reported similar Fe concentrations in the roots of control plants and those treated with aged nZVI, with lower Fe levels than those of plants exposed to fresh nZVI [45]. Moreover, plants respond to soil Fe availability through mechanisms that enhance Fe mobilization under Fe-deficient conditions and restrict Fe uptake when Fe is present in excess [31].

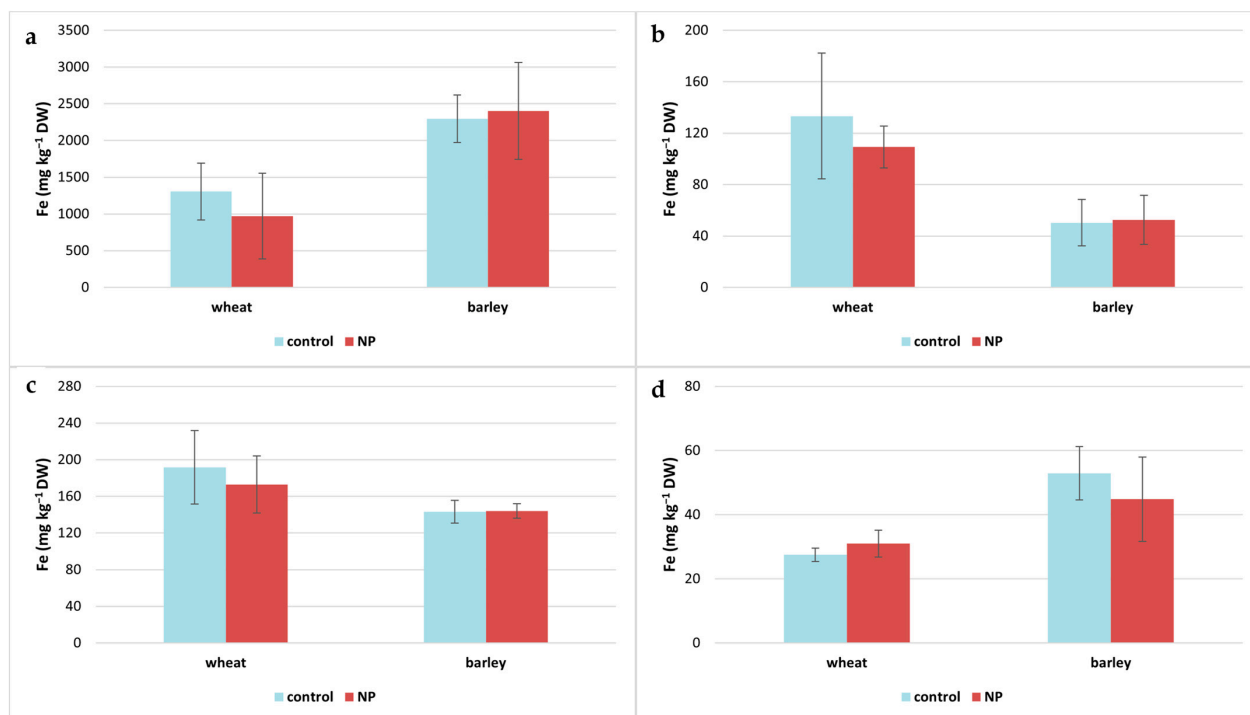


Figure 3. Iron concentration, g kg⁻¹, in different parts of barley (*Hordeum vulgare* L.) and wheat plants (*Triticum aestivum* L.), root (a), shoot (b), leaf (c), and grain (d). Data are presented as means and error bars indicate standard deviation ($n = 4$).

In addition, soil properties play a decisive role in modulating the impact of nZVI, as previously discussed. A 30-day experiment with lettuce grown in two soils with contrasting pH (acidic and alkaline), treated with the same commercial nZVI suspension (0.5 and 5%, equivalent to 0.8 and 8 g nZVI kg⁻¹), showed markedly different responses depending on soil physicochemical characteristics. In the acidic soil with low fertility, nZVI promoted plant development, increasing both biomass production and total Fe content, whereas under alkaline conditions, the effects were less pronounced, with a reduction in fresh biomass at the highest dose and an increase in Fe concentration detected only in the aerial tissues [41]. In near-neutral soil conditions, nZVI application—even at lower doses (0.3–1 g kg⁻¹)—leads to increased Fe concentrations in the roots of *V. radiata*, regardless of the specific nZVI type or dosage used. However, the translocation of Fe from roots to shoots appeared to be more selective, occurring only at the highest application rates and specifically when the nZVI was stabilized with starch or activated carbon [43]. An acidic soil treated with nZVI at 0.5 g kg⁻¹ also induced an increase in Fe concentration in both roots and shoots of four-week-old maize plants [48]. In summary, the treatment of an alkaline soil with nZVI under the experimental conditions tested did not stimulate Fe accumulation in barley or wheat tissues. Moreover, the two species showed distinct Fe accumulation patterns. Barley exhibited significantly higher Fe concentrations in roots and grains compared to

wheat, whereas wheat showed greater Fe accumulation in shoots and leaves. Systematic comparisons of iron translocation between barley and wheat remain limited.

The impact of the addition of nZVI to soil on Ca, K, Na, and Mg accumulation in different tissues of barley and wheat plants was also evaluated (Table 2), as little information is available on this topic. Overall, this analysis revealed species-specific responses to nZVI application. In particular, the treatment with nZVI resulted in a reduction in Ca accumulation, although this effect was statistically significant only in the roots and grains of barley, and in the shoots, leaves, and grains of wheat. Of note, higher Ca concentrations were observed in barley roots than in wheat roots in control plants, a trend that has also been reported in previous comparative studies [59,62]. The impact of nZVI on Mg accumulation was less pronounced than that observed for Ca; however, a significant reduction ($p < 0.05$) in Mg was detected in barley roots, as well as in the leaves and grains of wheat. Sodium increased in both species, particularly in barley. This was likely due to the stabilization of nZVI with a sodium salt, which also induced an increase in Na^+ availability in soil (Table 1). Previous studies involving the treatment of barley and lettuce plants with this type of nZVI also detected an increase in Na^+ in plant tissue, both in contaminated and uncontaminated soil [10,17,41]. Regarding K^+ , the shoots and grains of barley tended to show an increase when exposed to nZVI, whereas for wheat, significant differences were found only in leaves. Consistent with these findings, a previous experiment with barley grown in As-polluted soil and treated with nZVI also reported an increase in K^+ in plant tissue compared with the control [10]. Interactions among mineral elements can significantly affect nutrient bioavailability for root uptake. The acquisition of nutrients by plants depends not only on their availability in the soil solution but also on the capacity of roots to absorb and transport them [31]. Consequently, further studies are needed to elucidate the mechanisms underlying the interactions between Fe and other nutrients.

Table 2. Content of Ca, Mg, Na, and K (g kg^{-1} of dry weight) in roots, shoots, leaves, and grains of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) at the end of the cultivation. The data presented are the mean concentrations $\pm$ standard deviations ($n = 4$).

Species	Element	Treatment	Root	Shoot	Leaf	Grain
Wheat	Ca (g kg^{-1})	C	5.09 $\pm$ 0.94	3.55 $\pm$ 1.16 *	13.3 $\pm$ 1.24 ***	0.39 $\pm$ 0.03 ***
		NP	5.35 $\pm$ 0.61	0.92 $\pm$ 0.44	7.73 $\pm$ 1.10	0.12 $\pm$ 0.02
	Mg (g kg^{-1})	C	1.15 $\pm$ 0.31 *	0.98 $\pm$ 0.08	4.16 $\pm$ 0.33 *	1.32 $\pm$ 0.02 **
		NP	0.81 $\pm$ 0.19	0.87 $\pm$ 0.14	3.38 $\pm$ 0.14	1.13 $\pm$ 0.07
	Na (g kg^{-1})	C	0.64 $\pm$ 0.14	2.25 $\pm$ 0.29	0.35 $\pm$ 0.07	0.029 $\pm$ 0.027
		NP	0.83 $\pm$ 0.37	1.56 $\pm$ 0.96	0.37 $\pm$ 0.13	0.080 $\pm$ 0.014
	K (g kg^{-1})	C	1.92 $\pm$ 0.78	23.9 $\pm$ 2.92	14.5 $\pm$ 0.89 ***	4.69 $\pm$ 0.21
		NP	3.20 $\pm$ 1.27	19.0 $\pm$ 3.09	20.0 $\pm$ 0.57	5.06 $\pm$ 0.51
Barley	Ca (g kg^{-1})	C	24.2 $\pm$ 11.4 *	1.26 $\pm$ 0.61	12.9 $\pm$ 0.45	0.28 $\pm$ 0.02 ***
		NP	5.29 $\pm$ 2.29	0.51 $\pm$ 0.08	10.1 $\pm$ 2.20	0.03 $\pm$ 0.01
	Mg (g kg^{-1})	C	2.29 $\pm$ 0.50 *	1.88 $\pm$ 0.16	3.39 $\pm$ 0.13	1.25 $\pm$ 0.06
		NP	1.12 $\pm$ 0.15	1.64 $\pm$ 0.08	3.29 $\pm$ 1.24	1.20 $\pm$ 0.04
	Na (g kg^{-1})	C	0.82 $\pm$ 0.33 *	2.72 $\pm$ 1.37 *	0.83 $\pm$ 0.14	0.04 $\pm$ 0.00 *
		NP	1.68 $\pm$ 0.64	5.01 $\pm$ 0.78	2.65 $\pm$ 0.82	0.17 $\pm$ 0.04
	K (g kg^{-1})	C	2.88 $\pm$ 0.42	18.3 $\pm$ 6.21 *	29.6 $\pm$ 2.05	5.71 $\pm$ 0.08 ***
		NP	3.51 $\pm$ 1.05	27.7 $\pm$ 2.97	31.5 $\pm$ 10.5	7.45 $\pm$ 0.15

Asterisks mean significant differences based on Student's *t*-test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Thus, considering the present results, together with the available literature, plant responses to nZVI treatment vary depending on multiple factors, including application dose, nZVI type and aging, plant species, duration of exposure, soil properties, and the presence of contaminants. Accordingly, no general rule can be established a priori, and case-specific

assessments are required before recommending soil amendment with nZVI. The improved plant performance observed in contaminated soil compared with uncontaminated soils suggests that the presence of contaminants may partially mask the intrinsic effects of nZVI on plants.

3. Materials and Methods

3.1. Soil Characteristics

An agricultural loam soil, sampled at a depth of 0–30 cm, was collected from a site in Alcalá de Henares (40.52803° N, 3.29041° W, Madrid). Bulk soil samples were air-dried and sieved (<4 mm) before analysis. The physicochemical properties of the soil (Table 3) were determined following the Spanish official methodology for soil analysis [41,63]. Briefly, pH and electrical conductivity were measured in a 1:2.5 soil-to-water ratio. Carbonates were measured using a Bernard calcimeter, and organic matter was determined by oxidation with potassium dichromate. Available P was measured using the Olsen method, while total N was analyzed by the Kjeldahl method. Exchangeable cations (Ca, Mg, Na and K) were extracted with 0.1 M ammonium acetate and quantified by flame atomic absorption spectrometry (FAAS) (AA240FS, Varian, Mulgrave, Victoria, Australia). The concentrations of pseudo-total metals were measured after acid digestion using a mixture of nitric and hydrochloric acids in a microwave digestion system (Multiwave GO, Anton Paar GmbH, Graz, Austria). Quantitation was then performed using FAAS. Finally, soil texture was determined using a Bouyoucos densimeter.

Table 3. Physicochemical properties of the soil used in the study (mean $\pm$ standard deviation, $n = 3$).

Parameter	Value
pH	8.2 $\pm$ 0.2
EC (dS m ^{−1})	0.33 $\pm$ 0.03
CaCO ₃ (%)	8.6 $\pm$ 0.8
N (%)	0.11 $\pm$ 0.04
OM (%)	1.11 $\pm$ 0.10
P (mg kg ^{−1})	25 $\pm$ 5
Ca (mg kg ^{−1})	2930 $\pm$ 70
Mg (mg kg ^{−1})	440 $\pm$ 30
Na (mg kg ^{−1})	75 $\pm$ 25
K (mg kg ^{−1})	395 $\pm$ 71
Cd (mg kg ^{−1})	<LQ
Cr (mg kg ^{−1})	17 $\pm$ 3
Zn (mg kg ^{−1})	62 $\pm$ 22
Fe (g kg ^{−1})	19.9 $\pm$ 0.2
Sand (%)	49 $\pm$ 5
Silt (%)	31 $\pm$ 4
Clay (%)	19 $\pm$ 1

3.2. Zero-Valent Iron Nanoparticles

A commercially stabilized aqueous slurry of nZVI, NANOFE 25S (NANO IRON, Rajhrad, Czech Republic), was used. This material contains 14–18% Fe(0), 2–6% iron oxides, and 3% of the surfactant sodium polyacrylic acid and is produced from nanosized ferrihydrite using a dry-phase method. These nZVI particles have been extensively characterized both by the supplier (<https://nanoiron.cz/en/products/zero-valent-iron-nanoparticles/nanofer-25s>, accessed on 8 July 2026) and in previous studies [8,12,64]. In brief, the suspension has a strongly alkaline pH (11–12). The nanoparticles exhibit an average particle size of

50 nm, a surface area of $20 \text{ m}^2 \text{ g}^{-1}$, with a regular spherical morphology and the presence of chain-like aggregates, and high colloidal stability, with a zeta potential of -31.8 mV .

3.3. Greenhouse Experiment

A greenhouse pot experiment was conducted using barley (*Hordeum vulgare* L.) cultivar Pedrezuela and wheat (*Triticum aestivum* L.) cultivar Albares (November 2024–June 2025). Seeds of both species were initially sown in the soil described in Section 2.1. In parallel, soil samples were treated with an nZVI suspension at 0 and 5% (*w/w*), equivalent to 0 and 0.8% of the dried product, or 0 and 8 g nZVI/kg, respectively (hereafter referred to as C and NP treatments). This dose was selected based on previous experiments performed both with contaminated and uncontaminated [8,9,17,41,65]. Soil (3.6 kg) was mixed and homogenized with nZVI in a plastic container and transferred to a 4 L ceramic pot. After allowing the soil and nZVI to interact for 72 h, two plants of barley or wheat at Zadoks stage 14 [66] were transplanted into the pots. Four replicates per treatment were included. At Zadoks stage 22, the pots were fertilized with 5 g per pot of NPK 15:15:15. They were then periodically irrigated with tap water during the experiment. The characteristics of the tap water are shown in Table S1. Upon completion of their growth cycle, the plants were harvested (Zadoks stage 87–90, 5 months). The ears of each plant were cut, and the rest of the aerial part was cut at soil level and separated into stems and leaves. The roots were washed first with tap water, then with distilled water, and dried in an oven at 80°C for 48 h to obtain the dry weight. Plants from the same pot were treated together. Greenhouse environmental conditions, including temperature and relative humidity, are shown in Figure S2.

3.4. Plant Analysis

Chlorophyll content was determined using a nondestructive method with a portable chlorophyll meter SPAD-502 (Minolta Co., Tokyo, Japan) [67]. The measurements were carried out on the flag leaf of the main shoot when plants reached Zadoks stages 22, 40, and 69 (sampling times S1, S2, and S3, respectively). Four measurements per plant were performed, and the average value for each leaf was recorded. The correlation between the total chlorophyll measured in the laboratory and the SPAD values was expressed as: $[\text{Chl}] (\text{mg m}^{-3}) = 15.68 \times \text{SPAD values} - 209.03$ [67]. The relative water content (RWC) and the concentration of malondialdehyde (MDA) as a biomarker of oxidative stress relative to lipid peroxidation were determined at Zadoks stages 22–23 and 40 (sampling times S1 and S2, respectively) following the protocol previously described by [59]. For both RWC and MDA, four measurements were obtained per pot, and the mean value was used for statistical analysis.

The impact of nZVI addition to the soil on the internal ultrastructure of plant roots and leaves was evaluated by transmission electron microscopy (TEM). Samples were collected when plants reached Zadoks stage 69–70 (sampling time S3). Segments of approximately 2 mm from the primary root were collected and fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 6 h at 4°C , and then washed three times with the same buffer. Samples were post-fixed for 1 h in 1% osmium tetroxide and dehydrated in a graded acetone series (30, 50, 70, 80, 90, 95, and 100%; 20 min per step). Specimens were embedded in Spurr's resin and sectioned at 60 nm with an ultramicrotome (Leica EM UC7, Leica Microsystems GmbH, Wetzlar, Germany). Ultrathin sections were examined with a JEOL JEM-1100 (Akishima, Tokyo, Japan) transmission electron microscope operated at 80 kV.

Dried roots, stems, leaves, and grains were ground and mineralized following the Spanish official methodology for plant analysis [17,63]. In brief, plant samples were ashed

in a muffle furnace at 580 °C overnight; the ashes were then boiled with 1 mL of HCl (37%) and 5 mL of Milli-Q water for 30 min, filtered, and diluted to 50 mL with Milli-Q water. The concentrations of Fe, Ca, Mg, Na, and K were determined by FAAS.

3.5. Soil Analysis

The impact of the nZVI treatment on the physicochemical properties of the soil was evaluated. After harvest, soil samples were collected from the pots, air-dried, and homogenized. Analyses of pH, electrical conductivity, organic matter, N, available P, and exchangeable nutrients (Ca, Mg, Na, and K) were performed following the methods previously described in Section 2.1. The availability of Fe was also evaluated using the DTPA method [68].

3.6. Statistical Analysis

Data were statistically analyzed using the IBM SPSS Statistics software for Windows (version 30.0.0.0, Armonk, NY, USA). A Student's *t*-test was performed to determine statistically significant differences between the nZVI-treated and untreated samples. Levene's test was applied to assess the homogeneity of variances. The two plants from each pot were processed together, resulting in four replicates per treatment.

4. Conclusions

In this study, the treatment of an alkaline soil with a nZVI suspension at 5% (8 g nZVI kg^{−1} soil) resulted in a significant increase in soil Fe availability. However, this increase did not translate into higher Fe accumulation in barley or wheat tissues at the end of the growth cycle, nor did it promote growth in either species. Therefore, under the conditions tested, this commercial nZVI suspension, suitable for soil remediation, did not exhibit a fertilizing effect in the uncontaminated alkaline soil. Notably, both crops exhibited greater sensitivity to nZVI during the early stages of development; however, these initial adverse effects were progressively alleviated as plant growth advanced, allowing both species to complete their growth cycles.

Comparative analysis revealed species-specific responses, particularly in terms of Fe and nutrient accumulation patterns and biomass production, highlighting the importance of species-specific evaluations when assessing the behavior of nZVI in uncontaminated soils. Such studies are essential to properly assess the agronomic viability and potential risks of using nZVI as a fertilizer. Further experiments assessing a range of nZVI doses across different soil types and their effects on iron accumulation rates in different plants over the full growing cycle are needed to properly determine the potential of nZVI as an iron fertilizer.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/stresses6030047/s1>, Figure S1: Wheat (left) and barley (right) plants after three months of being transplanted; Figure S2. Minimum, maximum, and average temperature (a) and relative humidity (b) recorded in the greenhouse during the experiment; Table S1: Main characteristics of the tap water used in the study.

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Abbreviations

The following abbreviations are used in this manuscript:

EC	Electrical conductivity
FAAS	Flame Atomic Absorption Spectroscopy
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
MDA	Malondialdehyde
nZVI	Nanoscale zerovalent iron
OM	Organic matter
TEM	Transmission Electron Microscopy
RWC	Relative Water Content

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