environments

MDPI

*Article*

# Biogenic ZnO Nanoparticles Effectively Alleviate Cadmium-Induced Stress in Durum Wheat (*Triticum durum* Desf.) Plants

**Eleonora Coppa [1,†], Giulia Quagliata [1,†], Samuela Palombieri [1], Chiara Iavarone [1], Francesco Sestili [1], Daniele Del Buono [2] and Stefania Astolfi [1,*]**

[1] Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, 01100 Viterbo, Italy; ecoppa@unitus.it (E.C.); giulia.quagliata@unitus.it (G.Q.); palombieri@unitus.it (S.P.); chiara.iavarone@studenti.unitus.it (C.I.); francescosestili@unitus.it (F.S.)

[2] Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università di Perugia, 06123 Perugia, Italy; daniele.delbuono@unipg.it

* Correspondence: sastolfi@unitus.it; Tel.: +39-0761-357337

† These authors contributed equally to this work.

**Abstract:** This study investigated the potential of biogenic ZnO nanoparticles (ZnO-NPs) to alleviate cadmium (Cd) toxicity in durum wheat plants exposed for 14 days to 25 µM $CdSO_4$. By applying ZnO-NPs at two different concentrations (25 and 50 mg $L^{-1}$), we observed increased chlorophyll content, beneficially impacting the photosynthetic efficiency, and enhanced sulfur, zinc, and iron accumulation. Moreover, the ZnO-NP treatment reduced the Cd accumulation in shoots, mitigating leaf chlorosis and oxidative damage. This response was clearly mediated by the increased thiol and phytochelatin production, as well as the enhanced sulfate uptake rate, with *TdSultr1.3* as the most responsive gene coding for high-affinity transporter to Cd stress. In conclusion, the application of biogenic ZnO-NPs appears to be a promising approach for reducing the uptake of heavy metals by plants. In addition, it could be successfully used in combination with contamination prevention measures and/or remediation of contaminated sites to remove and mitigate the harmful effects of Cd on the environment and human health.

**Keywords:** *Lemna minor* extract; lipid peroxidation; nanostimulant; sulfur nutrition; thiol compounds



**Citation:** Coppa, E.; Quagliata, G.; Palombieri, S.; Iavarone, C.; Sestili, F.; Del Buono, D.; Astolfi, S. Biogenic ZnO Nanoparticles Effectively Alleviate Cadmium-Induced Stress in Durum Wheat (*Triticum durum* Desf.) Plants. *Environments* **2024**, *11*, 285. https://doi.org/10.3390/environments11120285

Academic Editor: Joaquim Esteves Da Silva

Received: 12 November 2024
Revised: 6 December 2024
Accepted: 9 December 2024
Published: 12 December 2024



## 1. Introduction

The degradation of arable soil is a significant environmental concern with broad implications for agriculture, ecosystems, and food security. The factors leading to soil degradation and compromising its quality, fertility, ecosystem services, and overall productivity are different, such as improper land use, deforestation, excessive chemical inputs, salinization, erosion, and climate change [1]. Soil contamination with heavy metals is a global issue of cogent concern that clashes with one of the greatest challenges of this century: how to meet the increasing demand for food with degraded or limited water and land availability [2].

Among heavy metals, cadmium is considered one of the most toxic, thus posing high threats to water and soil quality, food safety, and human health [3–5]. The accumulation of Cd in soils can be attributed to several sources, both natural and related to anthropogenic activities, with the former accounting for 10% compared to the total release from all the sources [6]. The main contributors to Cd contamination of agricultural soil are industrial activities, such as battery and pigment production, electroplating, and improper waste disposal. In addition, agriculture contributes to Cd release into land and soil due to the massive use of phosphate fertilizers, which can contain this metal as impurity and sewage sludge [7].

The presence of Cd in the soil negatively impacts plant development, leading to reduced or stunted growth, chlorosis, oxidative impairments, and, ultimately, plant death [8]. Cd is taken up by plant roots and translocated to aboveground organs, where it hinders metabolic processes [9]. Once accumulated in the edible parts of crops, it can enter the trophic chain through the consumption of contaminated food and water.

The accumulation of Cd in plant tissues stimulates the production of high levels of reactive oxygen species (ROS), leading to oxidative stress and, consequently, damage to the cellular components [10]. To mitigate or prevent these toxic effects, plants can activate the antioxidant defensive system, which involves the tripeptide glutathione (GSH) [11], and synthesize phytochelatins, which are small cysteine-rich peptides capable of chelating metal ions and sequester them into vacuoles [12,13]. Both GSH and phytochelatins synthesis require high sulfur (S) availability. For the above, when plants are exposed to Cd, the S uptake and assimilation rate are significantly stimulated [14–18].

High Cd levels in the soil can significantly interfere with the uptake of other nutrients due to the ability of this element to alter and modify cell wall permeability [19–21], as well as to compete with essential nutrients [22]. In fact, Cd can enter root cells through transmembrane transporters involved in the uptake of some divalent cationic nutrients, such as magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), and copper (Cu) [13,23].

Addressing the issue of soil contamination with Cd requires a holistic approach that integrates robust prevention measures and eco-friendly land management practices. Among the latter, besides sustainable remediation technologies, using hyperaccumulator plants and/or microorganisms to clean Cd-contaminated soils [24], the implementation of innovative strategies aimed at reducing Cd uptake by plants could play a crucial role in mitigating its harmful effects on food production and safety and human health.

In this context, nanomaterials appear to be one of the most promising and cutting-edge strategies to revolutionize agriculture, deliver benefits in crops, and, among others, mitigate the impacts of soil Cd contamination [25]. By definition, nanomaterials are composed of particles that show at least one dimension in the 1–100 nm range [26]. Many different nanostructured materials have been designed and developed, and they can be, depending on their composition, organic, inorganic, hybrid systems, or composites [26,27]. However, the use of these materials in agriculture is still considered to be in the initial stages, even though nanotechnology is expected to gain a groundbreaking role in the coming years in improving cropping systems and their sustainability [26,28,29].

Despite this, agriculture has already implemented some nanostructured materials such as nanopesticides, nanofertilizers [30], and plant nanobiostimulants. For instance, the benefits of nanostructured materials in promoting plant performance under stressful and challenging conditions have already been documented [31]. Indeed, it has been demonstrated that applying ZnO-NPs in plants can mitigate the negative impact caused by Cd and other heavy metals [32,33].

Moreover, it is necessary to point out that there is an increasing interest in biofabricated metal oxide nanoparticles, as they can be obtained by a simple bottom-up approach based on using biological extracts that, given their composition, can operate as capping or reducing agents, allowing for obtaining very small nanomaterials and controlling their shape and morphology [34,35]. Biogenic ZnO-NPs have been widely applied to plants with positive effects on biomass production, chlorophyll, and carotenoid content [36]. Moreover, the foliar application of ZnO-NPs to wheat plants [33] and *Leucaena leucocephala* (Lam.) de Wit [37], besides stimulating plant growth and chlorophyll content, has been shown to enhance Zn accumulation while reducing the uptake of Cd and Pb, leading to decreased oxidative damage in the shoot tissues. This reduction in Cd content was attributed, at least in part, to the chemical–physical similarity and analogous biological interaction mechanisms of Zn and Cd [38]. It has indeed been reported that Zn foliar application reduced the Cd concentration in rice plants [39] and mitigated the Cd toxicity in wheat [40]. Although the efficacy of biogenic ZnO-NPs in nutrient uptake, especially Zn, has been well recognized, their role in Cd toxicity mitigation remains unclear.

From such premises, this research aimed to investigate whether biogenic ZnO-NPs, obtained through applying a biobased synthesis involving an extract from *Lemna minor* L. [34], could act as nanobiostimulant in durum wheat (*Triticum durum* Desf., cv. Svevo) and then to understand the potential of the application of these NPs in crop production. Furthermore, the ZnO-NPs' potential in mitigating the harmful effects of Cd on durum wheat plants by limiting the uptake of this pollutant is discussed. To this scope, plants were grown in the presence or absence of Cd (25 and 0 μM $CdSO_4$, respectively), and ZnO-NPs were applied to plants by foliar spraying at two different concentrations.

## 2. Materials and Methods

### *2.1. Biogenic Synthesis of ZnO-NPs*

The synthesis of ZnO-NPs was conducted by following a previously developed biogenic protocol [34] based on the use of a hydroalcoholic extract obtained from *Lemna minor* L. This species was chosen to bio-fabricate NPs for its metabolic signature characterized by untargeted metabolomics [34], which allows it to act as a capping agent. The biogenic ZnO-NPs showed a morphology of spherical shape and sizes distributed in the range of 10–20 nm (all characterization data are reported in Del Buono et al. [34].

### *2.2. Plant Growth Conditions*

The seedlings of durum wheat (*Triticum durum* Desf., cv. Svevo) were grown in a growth chamber in hydroponic culture, with six plants per pot containing 2.2 L of nutrient solution (NS) [41]. The growth conditions were 27/20 °C with 14/10 h day/night cycles and relative humidity of 80% and 200 μmol $m^{-2}$ $s^{-1}$ PAR at leaf level. Seven days after sowing, Cd was added to the NS as $CdSO_4$ to half of the plants at a concentration of 25 μM. After 2 and 10 days from Cd-contamination, ZnO-NPs synthesized according to Del Buono et al. [34] were applied by foliar treatment (2 mL per plant) at two different concentrations, 25 and 50 mg $L^{-1}$ (in the text is referred to as 25 and 50 ppm). These ZnO-NP concentrations were selected based on the prior research [34], indicating their beneficial effects on the growth rate of maize seedlings under non-stressful conditions. At harvest (two weeks after Cd contamination), the plants were divided into six groups, as described in Table 1, based on the presence of $CdSO_4$ in the NS (0 and 25 μM) and the ZnO-NPs concentration (25 or 50 ppm).

**Table 1.** Growth conditions based on the concentration of Cd in the NS and of ZnO-NPs for the foliar treatments.

| *Treatments* | *CD [μM]* | *ZnO-NPs [ppm]* |
|---|---|---|
| **C** | 0 | 0 |
| **C25** | 0 | 25 |
| **C50** | 0 | 50 |
| **CD** | 25 | 0 |
| **CD25** | 25 | 25 |
| **CD50** | 25 | 50 |

### *2.3. Chlorophyll Content*

The chlorophyll content per unit area was assessed in the attached leaves by means of a SPAD portable apparatus (Minolta Co., Osaka, Japan), with measurements taken on the first fully expanded leaf from the top of each plant. The measurements were carried out at harvest (21 days after sowing and 4 days after the second ZnO-NPs treatment).

### *2.4. Determination of Malondialdehyde (MDA) Concentration*

The concentration of malondialdehyde (MDA) was determined as thiobarbituric acid (TBA) reactive metabolites, following the method outlined by [42]. In brief, fresh shoot and root tissues (0.2 g) were homogenized in 10 mL of 0.25% TBA prepared in 10% trichloroacetic

acid (TCA). The resulting extract was heated at 95 °C for 30 min and then rapidly cooled on ice. The suspension was centrifuged at 10,000× *g* for 10 min, and the absorbance of the supernatant was recorded at 532 nm. Non-specific turbidity was corrected by subtracting the absorbance value taken at 600 nm. The level of lipid peroxidation was quantified as mmol per gram of fresh weight using an extinction coefficient of 155 mM $cm^{-1}$.

### *2.5. Determination of Concentration of Non-Protein Thiol Compounds*

The quantification of water-soluble non-protein thiol compounds was performed colorimetrically using 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB), following the method described by [42]. Briefly, frozen shoot and root tissues (1 g FW) were homogenized in a solution (5 mL) containing 80 mM trichloroacetic acid (TCA), 1 mM ethylenediaminetetraacetic acid (EDTA), 0.15% (*w*/*v*) ascorbic acid and 10% (*w*/*v*), and polyvinylpyrrolidone (PVP). After a centrifugation step (30 min at 4000× *g* and 4 °C), the supernatants were collected, and the concentrations of DTNB-reactive compounds were detected spectrophotometrically at 415 nm (Agilent Cary 3500 UV-Vis Spectrophotometer, Santa Clara, CA, USA).

### *2.6. Determination of S, Zn, Fe, and Cd Concentrations*

To assess the total S concentration, the shoot and root tissues (1 g) were oven-dried at 105 °C and then ashed in a muffle furnace at 600 °C. Subsequently, the ashes were completely mineralized and dissolved in 10 mL of 3 N HCl, followed by filtration through Whatman No. 42 papers. Upon contact with $BaCl_2$, a $BaSO_4$ precipitate was formed and quantified using the turbidimetric method of Bardsley and Lancaster [43].

To assess the concentration of Zn, Fe, and Cd, the shoot and root tissues were dried at 121 °C for 24 h and then mineralized in 3 mL of ultrapure $HNO_3$ (69.5%) and 0.6 mL of HCl (37%) using a Microwave digestion system (Multiwave Go Plus, Anton Paar GmbH, Graz, Austria). After mineralization, the samples were filtered and diluted 1:200 with Milli-Q water. The concentrations of the nutrients were measured using an inductively coupled plasma-mass spectroscopy (Agilent 7850 ICP-MS, Santa Clara, CA, USA).

### *2.7. Gene Expression Analysis*

The total RNA was extracted from the shoot and root of plants grown in the presence or absence of 25 µM Cd using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO, USA). To synthesize single-strand cDNA, 1 µg of RNA was processed with the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). Quantitative real-time PCR (qRT-PCR) was performed on a CFX 96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Reactions were set up in a total volume of 15 µL, including 7.5 µL of SsoAdvUniver SYBR GRN SMX (Bio-Rad), primers at a concentration of 0.5 µM each, and 1 µL of cDNA. The thermal cycling protocol, based on Sestili et al. [44], consisted of an initial denaturation at 94 °C for 30 s, 40 cycles of denaturation at 94 °C for 5 s, annealing at 60 °C for 30 s, and a melting curve analysis from 65 °C to 95 °C, increasing by 0.5 °C every 5 s. The β-actin gene served as the internal control. Gene expression was quantified using the $2^{-\Delta\Delta Ct}$ method [45]. The primers were specifically designed to target the two high-affinity sulfate transporters (*TdSultr1.1* and *TdSultr1.3*), as described in Ciaffi et al. [46]. The experimental data were obtained from three independent biological replicates per genotype, with each biological replicate analyzed in triplicate for technical consistency.

### *2.8. Statistical Analysis*

The results are presented as mean ± standard deviation (SD) of three biological replicates (n = 3) for each analyzed parameter. The six different growth conditions were statistically analyzed using one-way analysis of variance (ANOVA) with a Student's *t*-test at $p < 0.05$ significance level using the statistical software CoStat (version 6.45). The correlation networks were performed in R (version 4.2.3) using the following packages: "tidyverse" (version 2.0.0), "corrr" (version 0.4.4), "igraph" (version 1.5.1), and "ggraph" (version 2.1.0).

## 3. Results

### *3.1. Biomass Production and Chlorophyll Content*

The foliar application of ZnO-NPs did not significantly alter plant biomass, as measured by the fresh weight of both shoots and roots (Figure 1). On the other hand, the Cd exposure (Cd condition) resulted in a significant decrease in both the shoot and root biomass (83% and 88%, respectively, compared to the control condition) (Figure 1). While the ZnO-NPs treatment partially mitigated the negative impact of Cd on shoot biomass, it had no significant effect on root biomass (Figure 1). Accordingly, at harvest, the shoot biomass of plants from the Cd25 and Cd50 conditions significantly increased by 59% and 79%, respectively, compared to those from the Cd condition (Figure 1).

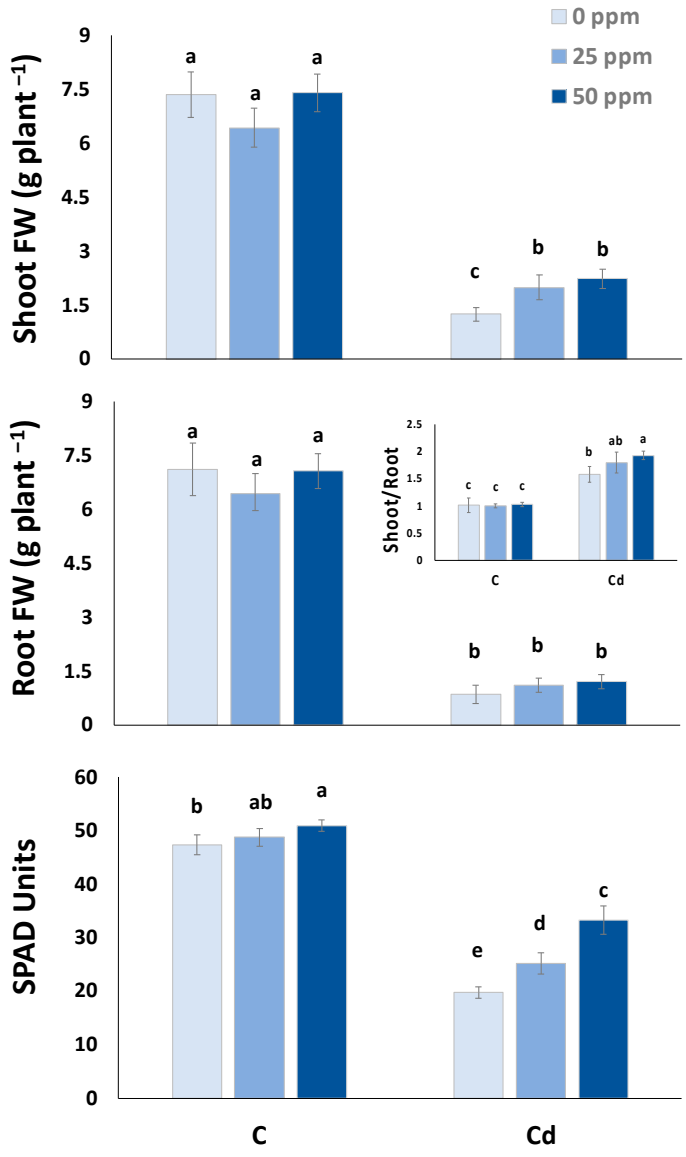


**Figure 1.** Effect of foliar application of ZnO-NPs at the concentration of 0, 25, and 50 ppm on shoot and root biomass (FW, fresh weight), shoot-to-root ratio (insert), and chlorophyll content, measured as SPAD units, of durum wheat plants grown in the absence (C) or presence (Cd) of 25 µM Cd. Data are reported as the mean of three biological replicates ± SD (n = 3). Different lower case letters indicate statistically significant differences among the growth conditions ($p < 0.05$).

The presence of Cd in the NS significantly influenced the allocation of resources within the plant. In particular, the shoot/root ratio increased significantly in the Cd, Cd25, and Cd50 conditions by 56%, 79%, and 88%, respectively, compared to the control samples

(insert in Figure 1). In addition, the application of ZnO-NPs resulted in a significant increase in the shoot/root ratio by 14% and 22%, respectively, in the samples Cd25 and Cd50, compared to the condition with only Cd (Figure 1).

As shown in Figure 1, the application of the highest ZnO-NPs concentration (C50 condition) guaranteed slightly higher chlorophyll content (7% compared to the C condition). In contrast, the presence of Cd (Cd condition) in the NS reduced the chlorophyll content by 58% compared to the control (C condition) (Figure 1). However, the application of ZnO-NPs significantly improved the leaf chlorophyll content in plants exposed to Cd contamination. Specifically, we found that the ZnO-NPs' beneficial effects were dose-dependent. Indeed, the lowest concentration (Cd25 condition) and the highest concentration (Cd50 condition) resulted, respectively, in an increase of 28% and 68% compared to the Cd condition (Figure 1).

### *3.2. Oxidative Damage*

Malondialdehyde (MDA) is one of the most common biomarkers for assessing lipid peroxidation. Under control conditions, the application of NPs caused no significant changes in the MDA concentration in plant tissues at both doses applied (Figure 2). The plants subjected to Cd treatment alone (Cd condition) exhibited significantly higher levels of lipid peroxidation in both the shoot and root tissues (2.1- and 1.8-fold than the control C, respectively), indicative of cell membrane damage (Figure 2A,B). Interestingly, the NP foliar application after Cd exposure managed to sustain membrane integrity only at the shoot level, as shown by significantly lower MDA concentration in comparison with Cd-stressed plants not treated with NPs (Cd condition) (Figure 2A). In particular, the highest NP concentration was less effective in reducing MDA accumulation in shoots (−20 and −13% in the Cd25 and Cd50 conditions, respectively, compared to the Cd condition). In contrast, in the roots, both the NP concentrations further increased Cd-induced lipid peroxidation, as shown by significantly higher MDA levels compared to the Cd condition (25% and 57%, with the lowest and highest concentrations, respectively (Figure 2B).

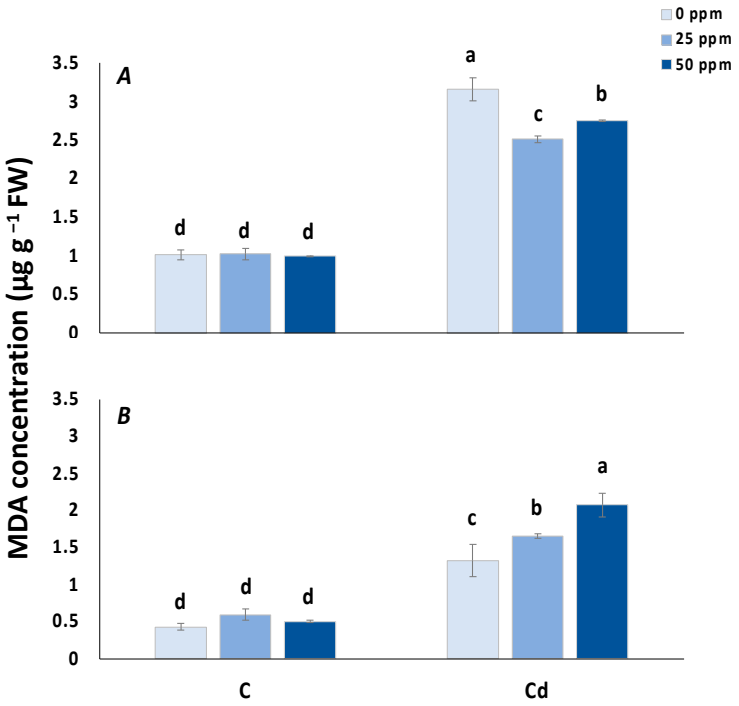


**Figure 2.** Oxidative damage expressed as changes in MDA accumulation in shoot (**A**) and root (**B**) tissues of durum wheat plants grown in the absence (C) or presence (Cd) of 25 µM Cd and treated foliarly with ZnO-NPs at the concentration of 0, 25, and 50 ppm. Statistics as in Figure 1.

### 3.3. Defensive Response

Neither low nor high doses of ZnO-NPs significantly altered the non-protein thiol concentrations in plants not exposed to cadmium (Cd) (Figure 3A,B). In contrast, Cd exposure (Cd condition) led to a significant increase in the non-protein thiol pool. In particular, the thiol concentration increased by 1.5- and 6.2-fold compared to the control (C), in shoots and roots, respectively (Figure 3A,B). The application of ZnO-NPs after Cd stress exposure was associated with a marked reduction in the thiol concentration in shoots in comparison to the samples treated with Cd alone (Cd 0) (Figure 3A). The trend recorded was dose-dependent, with the lowest dosage (Cd25 condition) resulting in a 66% decrease and the highest one (Cd50 condition) in an 84% decrease (Figure 3A). On the contrary, there was no same response at the root level as observed at the shoot level, as illustrated by significantly higher thiol accumulation in the roots of plants from the Cd25 and Cd50 conditions compared to the Cd-stressed plants (33 and 16%, respectively) (Figure 3B).

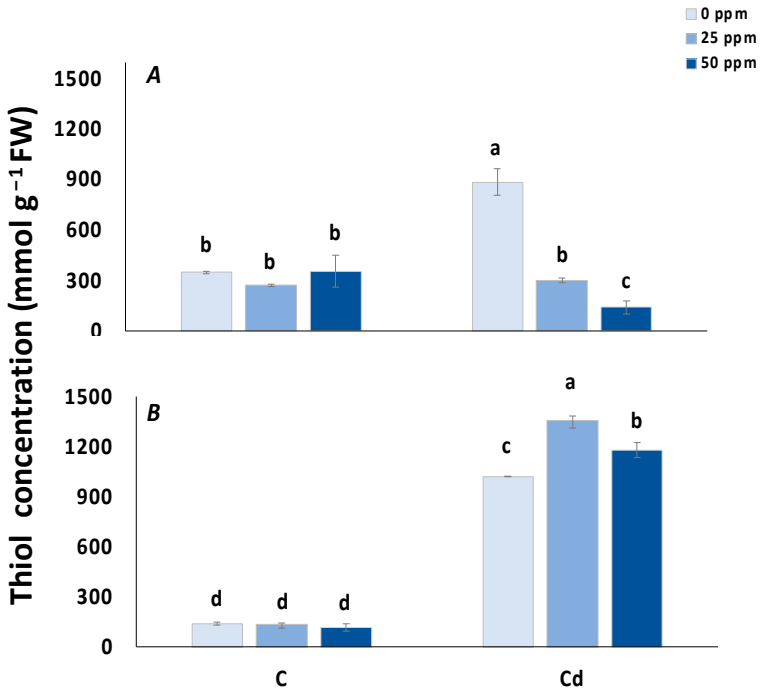


**Figure 3.** Effect of foliar application of ZnO-NPs at the concentration of 0, 25, and 50 ppm on non-protein thiols concentration in the shoot (**A**) and root (**B**) tissues of durum wheat plants grown in the absence (C) or presence (Cd) of 25 µM Cd. Statistics as in Figure 1.

### 3.4. Changes in Cd, Zn, and Fe Concentration

As expected, Cd exposure significantly increased the Cd accumulation in both the root and shoot tissues of wheat plants, with most of the Cd accumulated to a significantly higher extent in the roots than in the aboveground tissues (Figure 4). Interestingly, ZnO-NP application effectively reduced the Cd accumulation in the shoots by 81 and 79% compared to the Cd-stressed plants at 25 and 50 ppm, respectively (Figure 4). On the other hand, while the lower NP concentration (Cd25 condition) reduced the root Cd levels (37% compared to the corresponding control), the higher concentration had no significant effect (Figure 4). The ability of a plant to translocate Cd from the root system to the aerial parts is indicated by the translocation factor, calculated as the percentage ratio of shoot Cd concentration to root Cd concentration [47]. Importantly, ZnO-NP treatment also reduced the translocation of Cd from roots to shoots. The translocation rate decreased from 3% in the untreated Cd-exposed plants to 0.78% in Cd50 and 0.40% in Cd25 conditions (Figure 4).

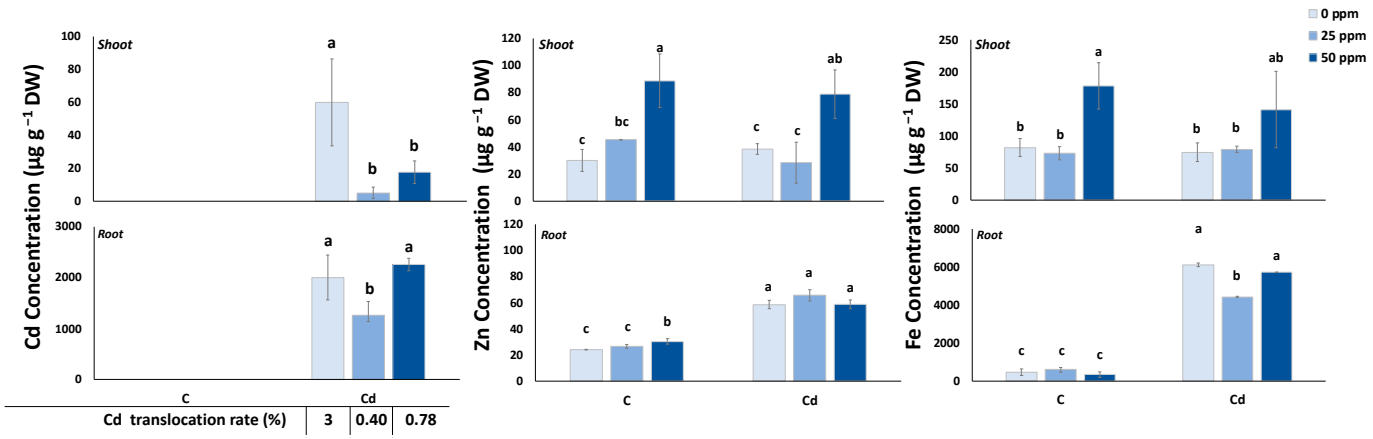


**Figure 4.** Changes in Cd, Zn, and Fe concentration (from left to right) in the shoot and root tissues of durum wheat plants grown in the absence (C) or presence (Cd) of 25 µM Cd and treated foliarly with ZnO-NPs at the concentration of 0, 25, and 50 ppm. The translocation rate, a measure of a plant's ability to move Cd from roots to shoots, was calculated as the percentage ratio of shoot-to-root Cd concentration. Statistics as in Figure 1.

Both the foliar application of ZnO-NPs and exposure to Cd affected the accumulation of two important micronutrients, Zn and Fe, in the plant tissues.

In plants from the control condition, the application of the highest NP concentration (C50 condition) caused a significant increase in Zn in both the shoot and root tissues but to a different extent. In particular, the C50 shoots exhibited a significantly higher (two-fold) Zn concentration than that found in C plants, whereas in roots, the increase was less pronounced (26% compared to control C) (Figure 4). The exposure of plants to Cd did not result in any significant variation in the Zn accumulation in the aboveground portion of the plants (Figure 4), while higher concentrations of Zn were detected in roots (144% compared to control C) (Figure 4). In Cd-stressed plants, a significant increase in the shoot Zn concentration was found following the application of the highest concentration of NPs (Cd50 condition) (Figure 4). The NP treatment promoted the accumulation of Zn in root tissue, but the peak was reached in the plants exposed to the lowest NP concentration (25 ppm) and was less pronounced than that observed in shoots (Figure 4).

Accumulation of Fe followed a pattern similar to that described for Zn, at least in shoots, with higher values in plants treated with the highest NP concentration, irrespective of the presence or absence of Cd in the growth medium (Figure 4). Furthermore, as for Zn, the presence of Cd in the NS did not influence the concentration of the nutrient (Figure 4). On the other hand, although no variations induced by NPs were detected in the control plants (C25 and C50 conditions), higher Fe accumulation was found in the roots of plants exposed to Cd (Figure 4).

### *3.5. Changes in Plant Total S Concentration and Expression of Two High-Affinity Sulfate Transporter (TdSultr1.1 and TdSultr1.3) in Root Tissues*

The ZnO-NP treatment had contrasting effects on the total S accumulation in shoots and roots (Figure 5). While it did not affect the shoot S levels, it significantly increased the root S levels by 55% at both NP doses (Figure 5). The Cd treatment by itself determined a notable decrease in S concentration in the aboveground part of wheat plants (−40% compared to control C) and an increase at the root level (+143% compared to control C) (Figure 5). The foliar application of ZnO-NPs to Cd-exposed plants caused no significant modulation of S accumulation in shoots while resulting in a significant decrease in root tissues (Figure 5). In the latter case, the trend recorded was dose-dependent, with the lowest dose (Cd25 condition) resulting in a 21% increase and the highest one (Cd50 condition) in a 36% increase in comparison with the Cd-stressed plants (Figure 5).

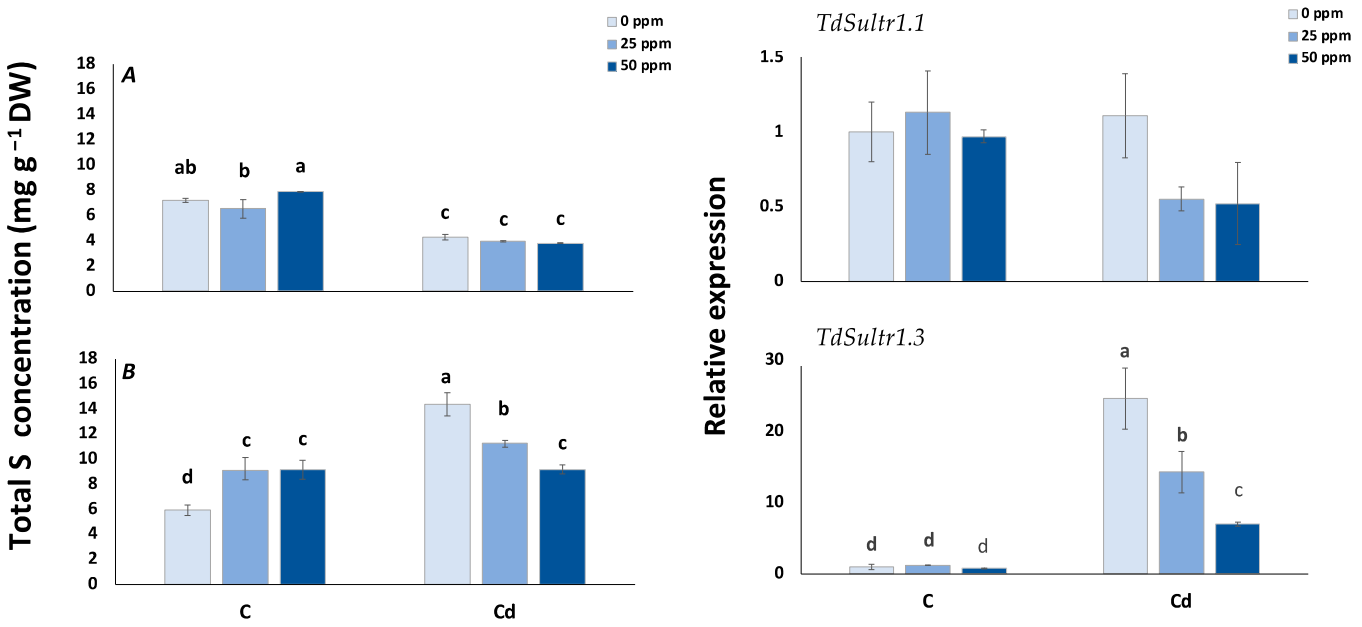


**Figure 5.** Effect of foliar application of ZnO-NPs at the concentration of 0, 25 and 50 ppm on total S concentration (**on the left**) in shoot (**A**) and root (**B**) tissues and on the relative expression levels by qRT-PCR of the genes encoding high-affinity sulfate transporters (*TdSultr1.1* and *TdSultr1.3*) (**on the right**) in roots of durum wheat plants grown in the absence (C) or presence (Cd) of 25 µM Cd. Statistics as in Figure 1.

In plants not exposed to Cd stress, the transcript levels of both high-affinity sulfate transporters belonging to group I (*TdSultr1.1* and *TdSultr1.3*) were not affected by the foliar application of ZnO-NPs, at both doses applied (Figure 5). The Cd treatment, with or without NP application, caused no significant modulation of *TdSultr1.1* expression but strongly upregulated *TdSultr1.3*, with the highest expression level detected in the roots of plants from the Cd condition (Figure 5).

### *3.6. Correlation Analysis*

The correlation networks, constructed using R software, revealed a higher degree of interconnection among the parameters in wheat shoots compared to roots, indicating a more complex physiological response in shoot tissues (Figure 6).

Interestingly, we found, in both the networks, a positive correlation between the total S concentration (S) and Cd translocation rate (TR) ($R^2$ = 0.929 and 0.8151, in shoots and roots, respectively) and a negative one between the ZnO-NPs concentration (ZnO.NPs) and Cd translocation rate (TR) ($R^2$ = 0.792 in both tissues) (Figure 6, Supplementary Table S1).

However, the two different networks also displayed specific features. For instance, the shoot network showed a strong positive correlation among the Cd translocation rate (TR) and MDA concentration (MDA) ($R^2$ = 0.972), MDA concentration (MDA) and thiols production (Thiols) ($R^2$ = 0.838), and thiols production (Thiols) and total S concentration (S) ($R^2$ = 0.999), suggesting that with the decreasing Cd translocation to shoots, the oxidative damage decreased, leading to lower demand of thiol compounds and then of sulfate (Figure 6A, Supplementary Table S1). Interestingly, the ZnO-NP concentration (ZnO.NPs) was positively correlated with both the chlorophyll levels (Chl) ($R^2$ = 0.994) and Fe accumulation (Fe) ($R^2$ = 0.895), and, as expected, a strong positive correlation was also found between the chlorophyll levels (Chl) and Fe accumulation (Fe) ($R^2$ = 0.939) (Figure 6A, Supplementary Table S1).

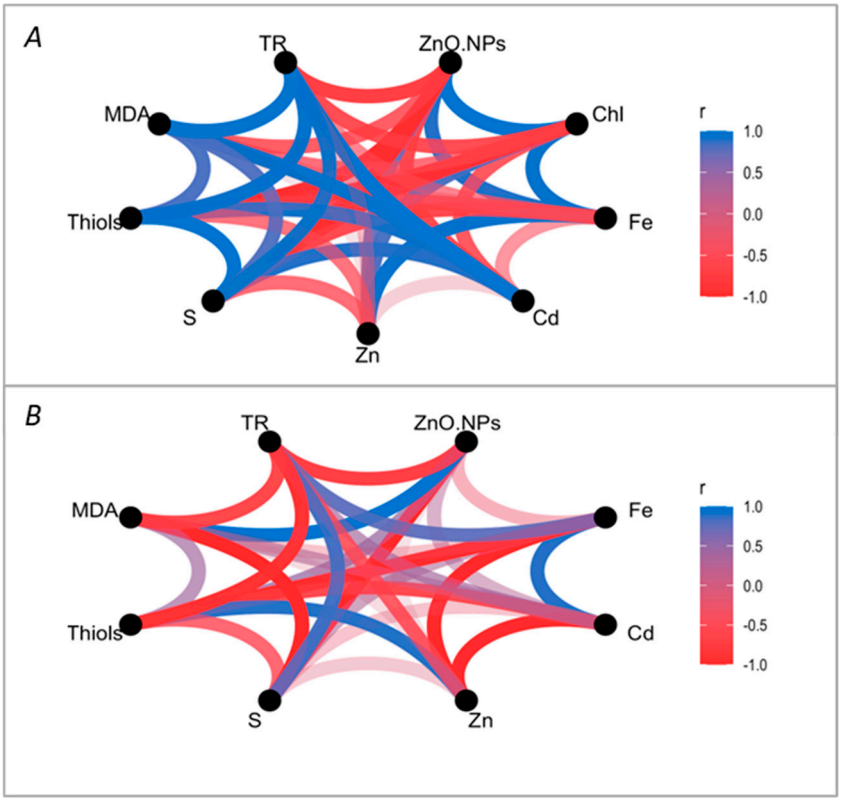


**Figure 6.** Correlation networks of durum wheat (**A**) shoots and (**B**) roots. The blue and red lines represent the positive and negative correlations, respectively. Abbreviations are as follows: Cd, cadmium concentration; Fe, iron concentration; Zn, zinc concentration; S, total S concentration; Thiols, non-protein thiols concentration; MDA, malondialdehyde concentration; TR, Cd translocation rate; and ZnO-NPs, the concentration of biogenic ZnO-NPs applied as foliar spray.

On the other hand, the root network revealed that the total S concentration (S) was negatively correlated with the MDA concentration (MDA) ($R^2$ = −0.985) (Figure 6B, Supplementary Table S1). Moreover, the Cd concentration in roots (Cd) was positively correlated with the Fe concentration (Fe) ($R^2$ = 0.892) but negatively correlated with the Zn concentration (Zn) ($R^2$ = −0.964) (Figure 6B, Supplementary Table S1).

## 4. Discussion

Industrial and mining activities, agricultural practices, and improper waste disposal are common sources of heavy metal pollution in soil. Impaired plant growth and development, reduced crop yields, and compromised food safety are the most obvious consequences of agricultural soil contamination with heavy metals [2]. Among heavy metals, Cd is one of the most harmful [3]; it can accumulate in agricultural soils and subsequently be taken up by crops [13], leading to a direct pathway of exposure for humans through the food chain [48,49].

To address this challenge, innovative strategies are needed, and one promising approach involves the utilization of nanotechnology. In this study, we explored the potential of bio-fabricated ZnO-NPs to alleviate the negative impacts of Cd on durum wheat. By employing the green synthesis method using a plant extract from *Lemna minor* [34], we aimed to develop eco-friendly and sustainable nanomaterials for agricultural applications.

It is well known that Cd negatively impacts plant development, leading to reduced growth and, ultimately, plant death [50]. Plants exposed to Cd commonly exhibit chlorotic symptoms due to the inhibition of key enzymes involved in chlorophyll biosynthesis, leading to reduced chlorophyll content and impaired light absorption, ultimately decreasing

the photosynthesis rate [51]. Accordingly, wheat plants exposed to Cd showed stunted growth and chlorosis, which are general symptoms of Cd toxicity [50].

The protective effect of ZnO-NPs on plants can occur through increased biomass, enhanced nutrient use efficiency, or increased resistance to stressful conditions, such as Cd in the growth medium. In fact, we found that the foliar application of biogenic ZnO-NPs was not effective in stimulating the shoot and root biomass production, but it slightly increased the chlorophyll content when plants were grown in control conditions and supplied with adequate availability of nutrients (Figure 1). This stimulatory effect induced by ZnO-NPs, even though ascertained at the highest dosage, can be attributed to the ability of this nanomaterial to influence the chlorophyll content in crops grown under both normal and stressed conditions [52]. Indeed, the involvement of Zn in the protochlorophyllide and chlorophyll biosynthesis, as well as chloroplast development, is well known [53]. In addition, ZnO-NPs, thanks to their electronic properties, may influence the ability of photosystem II to absorb light, thereby increasing the transmission of radiant energy from tyrosine to chlorophylls [54]. This mechanism, in turn, stimulates the chlorophyll biosynthesis to support the increased photosynthetic activity [54].

As for biomass production, the effect of NPs may depend on the species, the dosage, and the phenological stage at which the material is applied [26]. On the other hand, this pattern can be quite common and consistent with several results reported in the literature, showing that the beneficial effect of bioactive compounds occurs in plants exposed to limited resource availability (water and/or nutrients) or stress conditions [55]. In the latter case, it can be suggested that biostimulants could compensate for the growth rate during the adaptation to stress. Accordingly, Cd-induced inhibition of plant growth was, at least in part, mitigated by the foliar application of ZnO-NPs. At both concentrations, they alleviated the negative effects of Cd on shoot biomass without affecting root growth and thus resulted in an increased shoot/root ratio (Figure 1).

Furthermore, the treatment with ZnO-NPs significantly increased the leaf chlorophyll content in plants stressed with Cd, which, being positively correlated with NP concentration ($R^2$ = 0.933, Supplementary Table S1), indicated a dose-dependent response (Figure 1). The positive effect of ZnO-NPs on shoot growth and chlorophyll content might be ascribed to a lower uptake, translocation, and accumulation of Cd to shoots and/or by the activation of mechanisms to avoid the potentially damaging effects related to the onset of oxidative stress. In fact, following treatment with ZnO-NPs, not only did Cd accumulation in both shoot and root tissues significantly decrease, but its translocation rate to the shoot also decreased (Figure 4). Moreover, a positive correlation between the Cd translocation rate and Cd concentration in the shoots ($R^2$ = 0.996, Supplementary Table S1) was found, suggesting that the Cd translocation is likely the key driver of the Cd accumulation in the shoot, rather than the Cd uptake, according to the previous studies [56,57].

The reduction in the Cd uptake and accumulation induced by the foliar application of ZnO-NPs could be explained by the competitive mechanism between Zn and Cd, which, due to their similarity in terms of physicochemical properties, results in a similar interaction at the physiological level [38]. It has been indeed demonstrated that the Cd uptake and accumulation, along with its consequent phytotoxic effects, is inversely correlated with increasing Zn concentration in the growth medium [58–61].

As expected, the ZnO-NPs treatment, when applied at 50 ppm, led to an increased Zn accumulation in both the shoots and roots of wheat for both the control and Cd conditions (Figure 4). Interestingly, the increased accumulation of Zn in the shoots of plants exposed to Cd was associated with reduced Cd accumulation (Figure 4). Zn is an essential micronutrient for plants, playing a crucial role in various physiological and biochemical processes, such as the activation of numerous enzymes like RNA polymerase, superoxide dismutase, alcohol dehydrogenase, and carbonic anhydrase [62]. In addition to these functions, Zn plays an essential role in chloroplast development and some of their functions, such as the photosystem repair processes [63].

On the other hand, the positive effect of ZnO-NPs can be explained by assuming that it enhances the plant's ability to counteract the stressor. Therefore, the oxidative damage to lipids and the synthesis of antioxidant compounds in the root and shoots of wheat plants were evaluated. Cd exposure generally leads to increased production of ROS that can damage biological organelles and biomolecules, particularly by oxidizing the lipids of cell membranes [64]. The enhanced level of lipid peroxidation, expressed in terms of MDA concentration, observed in both the root and shoot tissues of Cd-exposed wheat plants are indicative of oxidative damage induced by the metal (Figure 2). Interestingly, a significant decrease in MDA accumulation was found in the shoot tissues of plants grown with Cd and treated with ZnO-NPs at both concentrations, revealing the potential role of these NPs in enhancing the plant's ability to face oxidative stress. However, the MDA concentration in roots did not appear to follow the same pattern, as it increased by increasing the ZnO-NPs dosage ($R^2$ = 0.998, Supplementary Table S1) (Figure 2). The occurrence of two different response patterns following the application of the ZnO-NPs may be attributed to the higher Cd concentrations found in roots, which corresponded to more than 30-fold that found in shoots. This finding is consistent with that seen in other plant species and can be considered a strategy for improving the Cd tolerance by limiting the Cd translocation to shoots [65,66].

The analysis of non-protein thiol compounds, which play a pivotal role as antioxidants in plants, being ROS scavengers [67], was conducted in both the root and shoot tissues. The non-protein thiols pool increased in plants treated with Cd compared to the control (C) in both shoot and root tissues, whereas it decreased after the application of ZnO-NPs in a dose-dependent manner ($R^2$ = −0.950, Supplementary Table S1) (Figure 3). The highest concentration of thiols found in roots was not surprising, as this part of the plant is the primary site for Cd accumulation.

There are several reports connecting Cd stress to changes in sulfur (S) uptake and assimilation rate [14,16,17] due to the increased demand for S to produce S-containing metabolites, such as thiols and phytochelatins (PCs), which are considered a protection against oxidative damage and the main defense mechanism, respectively [68]. Accordingly, the presence of Cd in the growth medium significantly increased the total S concentration in the roots while decreasing it in the shoots (Figure 5). This response reasonably accounts for the observed patterns of Cd accumulation, thiol production, and MDA accumulation, which occurred mainly in the roots.

In addition, the total S levels in the roots were affected by the treatment with ZnO-NPs but in different ways, depending on whether the plants were exposed to Cd. In particular, the application of ZnO-NPs to wheat under control conditions led to a significant increase in the S concentration but to a lesser extent compared with plants exposed to Cd. In contrast, S decreased in a dose-dependent manner under the Cd condition ($R^2$ = −0.994, Supplementary Table S1) (Figure 6). The latter result was consistent with the expression pattern of the *TdSultr1.3* gene, encoding a high-affinity sulfate transporter involved in the re-distribution of S from source to sink organs [69] (Figure 5). The expression of genes encoding sulfate transporters is regulated by sulfur supply, with reduced external S availability, leading to their rapid upregulation [70]. In addition, environmental factors, such as external Cd, can modulate the demand for S, driving plants to adapt their S uptake rate [71,72]. Our finding demonstrates that between the two analyzed high-affinity sulfate transporters, *TdSultr1.1* and *TdSultr1.3*, the latter had a crucial role in matching the higher S demand needed to cope with Cd stress by increasing the sulfate uptake capacity of the roots. Indeed, Cd treatment, with or without ZnO-NPs application, caused no significant modulation of the expression of *TdSultr1.1*, which has been shown to be the most responsive gene to S [46].

It is well known that the presence of Cd in the external medium could affect the uptake of other nutrients, resulting in either increased, as previously observed for S, or reduced uptake, as previously demonstrated for Fe in barley [66] and tobacco [18] plants. The uptake of Cd through transmembrane transporters belonging to the ZIP (ZRT, IRT-like protein) and Nrampm (Natural resistance-associated macrophage protein) transporter families, usually

used by some divalent nutrients, is considered a likely reason for the second mechanism leading to competition phenomena. Given that Fe is a micronutrient of extreme importance for plant nutrition, and to shed light on these antagonizing dynamics, the patterns of Fe accumulation in both the roots and shoots of plants subjected to various treatments were analyzed. Our experiment pointed out that the highest values for Fe were found in plants treated with 50 ppm ZnO-NPs, irrespective of the Cd presence in the medium. This result suggests a potential contribution of ZnO-NPs in favoring Fe acquisition as a compensatory mechanism activated by this material in plants grown under Cd stress (Figure 4). On the other hand, the Fe accumulation in roots was highest in plants exposed to Cd, according to the previous studies on Arabidopsis roots [18], and this result ruled out the possibility of a possible competitive effect.

In conclusion, this study demonstrated that the bio-fabrication of ZnO-NPs using plant extracts obtained from biological entities readily available in nature, such as *Lemna minor*, can represent an effective tool in counteracting heavy metal uptake in crops. In fact, the findings of this study demonstrate that the application of biogenic ZnO-NPs effectively mitigated the Cd uptake and translocation in wheat plants, thereby reducing its detrimental impact on the crop. From that perspective, this study enables new applications of bio-based nanomaterials for the mitigation potential they may show toward certain pollutants and their potential for use with other natural-based solutions to recover or protect non-renewable resources.

Finally, our research highlights the potential benefits of nanomaterials in agriculture. However, to harness these benefits responsibly, a thorough assessment of their environmental impact and potential risks to plant health is crucial [73].

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments11120285/s1, Table S1: The correlation coefficients and significance levels between two sets of elements in the durum wheat plants.

**Author Contributions:** Writing—original draft, investigation, and formal analysis were performed by E.C. Investigation and formal analysis were performed by G.Q. and C.I. Investigation, formal analysis, and writing—review and editing were performed by S.P. and F.S. Writing—review and editing and conceptualization were performed by D.D.B. Writing—review and editing, conceptualization, validation, supervision, resources, and funding acquisition were performed by S.A. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** The data generated and analyzed during the current study are available from the corresponding author upon reasonable request.

**Acknowledgments:** Progetto ECS 0000024 Rome Technopole—CUP B83C22002820006, PNRR Missione 4 Componente 2 Investimento 1.5, funded by European Un-ion—NextGenerationEU.

**Conflicts of Interest:** All authors declare they have no competing interests.

## References

1. Kopittke, P.M.; Minasny, B.; Pendall, E.; Rumpel, C.; Mc Kenna, B.A. Healthy Soil for Healthy Humans and a Healthy Planet. *Crit. Rev. Environ. Sci. Technol.* **2023**, *54*, 210–221. [CrossRef]
2. Mora, O.; Le Mouël, C.; de Lattre-Gasquet, M.; Donnars, C.; Dumas, P.; Réchauchère, O.; Brunelle, T.; Manceron, S.; Marajo-Petitzon, E.; Moreau, C.; et al. Exploring the Future of Land Use and Food Security: A New Set of Global Scenarios. *PLoS ONE* **2020**, *15*, e0235597. [CrossRef] [PubMed]
3. Rizwan, M.; Ali, S.; Adrees, M.; Ibrahim, M.; Tsang, D.C.W.; Zia-ur-Rehman, M.; Zahir, Z.A.; Rinklebe, J.; Tack, F.M.G.; Ok, Y.S. A Critical Review on Effects, Tolerance Mechanisms and Management of Cadmium in Vegetables. *Chemosphere* **2017**, *182*, 90–105. [CrossRef] [PubMed]
4. Khan, M.A.; Khan, S.; Khan, A.; Alam, M. Soil Contamination with Cadmium, Consequences and Remediation Using Organic Amendments. *Sci. Total Environ.* **2017**, *601–602*, 1591–1605. [CrossRef] [PubMed]
5. Rezapour, S.; Atashpaz, B.; Moghaddam, S.S.; Damalas, C.A. Heavy Metal Bioavailability and Accumulation in Winter Wheat (*Triticum aestivum* L.) Irrigated with Treated Wastewater in Calcareous Soils. *Sci. Total Environ.* **2019**, *656*, 261–269. [CrossRef]

6. Pan, L.; Ma, J.; Wang, X.; Hou, H. Heavy Metals in Soils from a Typical County in Shanxi Province, China: Levels, Sources and Spatial Distribution. *Chemosphere* **2016**, *148*, 248–254. [CrossRef]
7. Mubeen, S.; Ni, W.; He, C. Agricultural Strategies to Reduce Cadmium Accumulation in Crops for Food Safety. *Agriculture* **2023**, *13*, 471. [CrossRef]
8. Jawad Hassan, M.; Ali Raza, M.; Ur Rehman, S.; Ansar, M.; Gitari, H.; Khan, I.; Wajid, M.; Ahmed, M.; Abbas Shah, G.; Peng, Y.; et al. Effect of Cadmium Toxicity on Growth, Oxidative Damage, Antioxidant Defense System and Cadmium Accumulation in Two Sorghum Cultivars. *Plants* **2020**, *9*, 1575. [CrossRef]
9. Haider, F.U.; Liqun, C.; Coulter, J.A.; Cheema, S.A.; Wu, J.; Zhang, R.; Wenjun, M.; Farooq, M. Cadmium Toxicity in Plants: Impacts and Remediation Strategies. *Ecotoxicol. Environ. Saf.* **2021**, *211*, 111887. [CrossRef]
10. Baig, N.; Ihsanullah; Sajid, M.; Saleh, T.A. Graphene-Based Adsorbents for the Removal of Toxic Organic Pollutants: A Review. *J. Environ. Manag.* **2019**, *244*, 370–382. [CrossRef]
11. Hasanuzzaman, M.; Nahar, K.; Anee, T.I.; Fujita, M. Glutathione in Plants: Biosynthesis and Physiological Role in Environmental Stress Tolerance. *Physiol. Mol. Biol. Plants* **2017**, *23*, 249–268. [CrossRef] [PubMed]
12. Sanità di Toppi, L.; Gabbrielli, R. Response to Cadmium in Higher Plants. *Environ. Exp. Bot.* **1999**, *41*, 105–130. [CrossRef]
13. Thomas, M. A Comparative Study of the Factors Affecting Uptake and Distribution of Cd with Ni in Barley. *Plant Physiol. Biochem.* **2021**, *162*, 730–736. [CrossRef] [PubMed]
14. Nocito, F.F.; Lancilli, C.; Crema, B.; Fourcroy, P.; Davidian, J.-C.; Sacchi, G.A. Heavy Metal Stress and Sulfate Uptake in Maize Roots. *Plant Physiol.* **2006**, *141*, 1138–1148. [CrossRef]
15. Astolfi, S.; Zuchi, S.; Neumann, G.A.; Cesco, S.; Sanità Di Toppi, L.; Pinton, R. Response of Barley Plants to Fe Deficiency and Cd Contamination as Affected by S Starvation. *J. Exp. Bot.* **2012**, *63*, 1241–1250. [CrossRef]
16. Lancilli, C.; Giacomini, B.; Lucchini, G.; Davidian, J.-C.; Cocucci, M.; Sacchi, G.A.; Nocito, F.F. Cadmium Exposure and Sulfate Limitation Reveal Differences in the Transcriptional Control of Three Sulfate Transporter (Sultr1;2) Genes in *Brassica juncea*. *BMC Plant Biol.* **2014**, *14*, 132. [CrossRef]
17. Yamaguchi, C.; Takimoto, Y.; Ohkama-Ohtsu, N.; Hokura, A.; Shinano, T.; Nakamura, T.; Suyama, A.; Maruyama-Nakashita, A. Effects of Cadmium Treatment on the Uptake and Translocation of Sulfate in *Arabidopsis thaliana*. *Plant Cell Physiol.* **2016**, *57*, 2353–2366. [CrossRef]
18. Lyčka, M.; Barták, M.; Helia, O.; Kopriva, S.; Moravcová, D.; Hájek, J.; Fojt, L.; Čmelík, R.; Fajkus, J.; Fojtová, M. Sulfate Supplementation Affects Nutrient and Photosynthetic Status of *Arabidopsis thaliana* and *Nicotiana tabacum* Differently under Prolonged Exposure to Cadmium. *J. Hazard. Mater.* **2023**, *445*, 130527. [CrossRef]
19. Dafré, A.L.; Sies, H.; Akerboom, T. Protein S-Thiolation and Regulation of Microsomal Glutathione Transferase Activity by the Glutathione Redox Couple. *Arch. Biochem. Biophys.* **1996**, *332*, 288–294. [CrossRef]
20. Ramos, I.; Esteban, E.; Lucena, J.J.; Gárate, A. Cadmium Uptake and Subcellular Distribution in Plants of Lactuca Sp. Cd–Mn Interaction. *Plant Sci.* **2002**, *162*, 761–767. [CrossRef]
21. Astolfi, S.; Zuchi, S.; Passera, C. Effect of Cadmium on H+ATPase Activity of Plasma Membrane Vesicles Isolated from Roots of Different S-Supplied Maize (*Zea mays* L.) Plants. *Plant Sci.* **2005**, *169*, 361–368. [CrossRef]
22. Hasan, S.A.; Fariduddin, Q.; Ali, B.; Hayat, S.; Ahmad, A. Cadmium: Toxicity and Tolerance in Plants. *J. Environ. Biol.* **2009**, *30*, 165–174. [PubMed]
23. Clemens, S. Toxic Metal Accumulation, Responses to Exposure and Mechanisms of Tolerance in Plants. *Biochimie* **2006**, *88*, 1707–1719. [CrossRef] [PubMed]
24. Raza, A.; Habib, M.; Kakavand, S.N.; Zahid, Z.; Zahra, N.; Sharif, R.; Hasanuzzaman, M. Phytoremediation of Cadmium: Physiological, Biochemical, and Molecular Mechanisms. *Biology* **2020**, *9*, 177. [CrossRef] [PubMed]
25. Faizan, M.; Bhat, J.A.; Hessini, K.; Yu, F.; Ahmad, P. Zinc Oxide Nanoparticles Alleviates the Adverse Effects of Cadmium Stress on Oryza Sativa via Modulation of the Photosynthesis and Antioxidant Defense System. *Ecotoxicol. Environ. Saf.* **2021**, *220*, 112401. [CrossRef]
26. Tolisano, C.; Del Buono, D. Biobased: Biostimulants and Biogenic Nanoparticles Enter the Scene. *Sci. Total Environ.* **2023**, *885*, 163912. [CrossRef]
27. Khalid, K.; Tan, X.; Mohd Zaid, H.F.; Tao, Y.; Lye Chew, C.; Chu, D.-T.; Lam, M.K.; Ho, Y.-C.; Lim, J.W.; Chin Wei, L. Advanced in Developmental Organic and Inorganic Nanomaterial: A Review. *Bioengineered* **2020**, *11*, 328–355. [CrossRef]
28. Fraceto, L.F.; Grillo, R.; de Medeiros, G.A.; Scognamiglio, V.; Rea, G.; Bartolucci, C. Nanotechnology in Agriculture: Which Innovation Potential Does It Have? *Front. Environ. Sci.* **2016**, *4*, 186737. [CrossRef]
29. Shang, Y.; Hasan, M.K.; Ahammed, G.J.; Li, M.; Yin, H.; Zhou, J. Applications of Nanotechnology in Plant Growth and Crop Protection: A Review. *Molecules* **2019**, *24*, 2558. [CrossRef]
30. Chhipa, H. Nanofertilizers and Nanopesticides for Agriculture. *Environ. Chem. Lett.* **2016**, *15*, 15–22. [CrossRef]
31. Rastogi, A.; Tripathi, D.K.; Yadav, S.; Chauhan, D.K.; Živčák, M.; Ghorbanpour, M.; El-Sheery, N.I.; Brestic, M. Application of Silicon Nanoparticles in Agriculture. *3 Biotech* **2019**, *9*, 90. [CrossRef] [PubMed]
32. Hussain, A.; Ali, S.; Rizwan, M.; Zia ur Rehman, M.; Javed, M.R.; Imran, M.; Chatha, S.A.S.; Nazir, R. Zinc Oxide Nanoparticles Alter the Wheat Physiological Response and Reduce the Cadmium Uptake by Plants. *Environ. Pollut.* **2018**, *242*, 1518–1526. [CrossRef] [PubMed]

33. Adrees, M.; Khan, Z.S.; Hafeez, M.; Rizwan, M.; Hussain, K.; Asrar, M.; Alyemeni, M.N.; Wijaya, L.; Ali, S. Foliar Exposure of Zinc Oxide Nanoparticles Improved the Growth of Wheat (*Triticum aestivum* L.) and Decreased Cadmium Concentration in Grains under Simultaneous Cd and Water Deficient Stress. *Ecotoxicol. Environ. Saf.* **2021**, *208*, 111627. [CrossRef] [PubMed]
34. Del Buono, D.; Di Michele, A.; Costantino, F.; Trevisan, M.; Lucini, L. Biogenic ZnO Nanoparticles Synthesized Using a Novel Plant Extract: Application to Enhance Physiological and Biochemical Traits in Maize. *Nanomaterials* **2021**, *11*, 1270. [CrossRef] [PubMed]
35. Kumar, R.; Verma, A.; Rakib, M.R.J.; Gupta, P.K.; Sharma, P.; Garg, A.; Girard, P.; Aminabhavi, T.M. Adsorptive Behavior of Micro(Nano)Plastics through Biochar: Co-Existence, Consequences, and Challenges in Contaminated Ecosystems. *Sci. Total Environ.* **2023**, *856*, 159097. [CrossRef]
36. Srivastav, A.; Ganjewala, D.; Singhal, R.K.; Rajput, V.D.; Minkina, T.; Voloshina, M.; Srivastava, S.; Shrivastava, M. Effect of ZnO Nanoparticles on Growth and Biochemical Responses of Wheat and Maize. *Plants* **2021**, *10*, 2556. [CrossRef]
37. Venkatachalam, P.; Jayaraj, M.; Manikandan, R.; Geetha, N.; Rene, E.R.; Sharma, N.C.; Sahi, S.V. Zinc Oxide Nanoparticles (ZnONPs) Alleviate Heavy Metal-Induced Toxicity in Leucaena Leucocephala Seedlings: A Physiochemical Analysis. *Plant Physiol. Biochem.* **2017**, *110*, 59–69. [CrossRef]
38. Tan, L.; Qu, M.; Zhu, Y.; Peng, C.; Wang, J.; Gao, D.; Chen, C. ZINC TRANSPORTER5 and ZINC TRANSPORTER9 Function Synergistically in Zinc/Cadmium Uptake 1. *Plant Physiol.* **2020**, *183*, 1235–1249. [CrossRef]
39. Zhen, S.; Shuai, H.; Xu, C.; Lv, G.; Zhu, X.; Zhang, Q.; Zhu, Q.; Núñez-Delgado, A.; Conde-Cid, M.; Zhou, Y.; et al. Foliar Application of Zn Reduces Cd Accumulation in Grains of Late Rice by Regulating the Antioxidant System, Enhancing Cd Chelation onto Cell Wall of Leaves, and Inhibiting Cd Translocation in Rice. *Sci. Total Environ.* **2021**, *770*, 145302. [CrossRef]
40. Dawar, K.; Mian, I.A.; Khan, S.; Zaman, A.; Danish, S.; Liu, K.; Harrison, M.T.; Saud, S.; Hassan, S.; Nawaz, T.; et al. Alleviation of Cadmium Toxicity and Fortification of Zinc in Wheat Cultivars Cultivated in Cd Contaminated Soil. *S. Afr. J. Bot.* **2023**, *162*, 611–621. [CrossRef]
41. Zhang, F.; Römheld, V.; Marschner, H. Role of the Root Apoplasm for Iron Acquisition by Wheat Plants. *Plant Physiol.* **1991**, *97*, 1302–1305. [CrossRef]
42. Quagliata, G.; Abdirad, S.; Celletti, S.; Sestili, F.; Astolfi, S. Screening of Triticum Turgidum Genotypes for Tolerance to Drought Stress. *Plant Physiol. Biochem.* **2023**, *194*, 271–280. [CrossRef]
43. Bardsley, C.E.; Lancaster, J.D. Determination of Reserve Sulfur and Soluble Sulfates in Soils. *Soil Sci. Soc. Am. J.* **1960**, *24*, 265–268. [CrossRef]
44. Sestili, F.; Garcia-Molina, M.D.; Gambacorta, G.; Beleggia, R.; Botticella, E.; De Vita, P.; Savatin, D.V.; Masci, S.; Lafiandra, D. Provitamin a Biofortification of Durum Wheat through a TILLING Approach. *Int. J. Mol. Sci.* **2019**, *20*, 5703. [CrossRef]
45. Livak, K.J.; Schmittgen, T.D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2-\Delta\Delta CT$ Method. *Methods* **2001**, *25*, 402–408. [CrossRef]
46. Ciaffi, M.; Paolacci, A.R.; Celletti, S.; Catarcione, G.; Kopriva, S.; Astolfi, S. Transcriptional and Physiological Changes in the S Assimilation Pathway due to Single or Combined S and Fe Deprivation in Durum Wheat (*Triticum durum* L.) Seedlings. *J. Exp. Bot.* **2013**, *64*, 1663–1675. [CrossRef] [PubMed]
47. Kalavrouziotis, I.K.; Rezapour, S.; Koukoulakis, P.H. Wastewater status in Greece and Iran. *Fresenius Environ. Bull.* **2012**, *22*, 11–21.
48. Rai, P.K.; Lee, S.S.; Zhang, M.; Tsang, Y.F.; Kim, K.H. Heavy Metals in Food Crops: Health Risks, Fate, Mechanisms, and Management. *Environ. Int.* **2019**, *125*, 365–385. [CrossRef] [PubMed]
49. Abedi, T.; Mojiri, A. Cadmium Uptake by Wheat (*Triticum aestivum* L.): An Overview. *Plants* **2020**, *9*, 500. [CrossRef]
50. Hernandez, L.; Cook, D.T. Modification of the Root Plasma Membrane Lipid Composition of Cadmium-Treated Pisum Sativum. *J. Exp. Bot.* **1997**, *48*, 1375–1381. [CrossRef]
51. Soni, S.; Jha, A.B.; Dubey, R.S.; Sharma, P. Mitigating Cadmium Accumulation and Toxicity in Plants: The Promising Role of Nanoparticles. *Sci. Total Environ.* **2024**, *912*, 168826. [CrossRef] [PubMed]
52. Adil, M.; Bashir, S.; Bashir, S.; Aslam, Z.; Ahmad, N.; Younas, T.; Asghar, R.M.A.; Alkahtani, J.; Dwiningsih, Y.; Elshikh, M.S. Zinc Oxide Nanoparticles Improved Chlorophyll Contents, Physical Parameters, and Wheat Yield under Salt Stress. *Front. Plant Sci.* **2022**, *13*, 932861. [CrossRef] [PubMed]
53. Salama, D.M.; Osman, S.A.; Abd El-Aziz, M.E.; Abd Elwahed, M.S.A.; Shaaban, E.A. Effect of Zinc Oxide Nanoparticles on the Growth, Genomic DNA, Production and the Quality of Common Dry Bean (*Phaseolus vulgaris*). *Biocatal. Agric. Biotechnol.* **2019**, *18*, 101083. [CrossRef]
54. Tryfon, P.; Sperdouli, I.; Adamakis, I.-D.S.; Mourdikoudis, S.; Moustakas, M.; Dendrinou-Samara, C. Impact of Coated Zinc Oxide Nanoparticles on Photosystem II of Tomato Plants. *Materials* **2023**, *16*, 5846. [CrossRef] [PubMed]
55. Dujmović, M.; Opačić, N.; Radman, S.; Fabek Uher, S.; Voća, S.; Šic Žlabur, J. Accumulation of Stinging Nettle Bioactive Compounds as a Response to Controlled Drought Stress. *Agriculture* **2023**, *13*, 1358. [CrossRef]
56. Anwen, X.; Danting, C.; Chin, L.W.; Zhihong, Y. Root Morphology and Anatomy Affect Cadmium Translocation and Accumulation in Rice. *Rice Sci.* **2021**, *28*, 594–604. [CrossRef]
57. Uraguchi, S.; Mori, S.; Kuramata, M.; Kawasaki, A.; Arao, T.; Ishikawa, S. Root-To-Shoot Cd Translocation via the Xylem Is the Major Process Determining Shoot and Grain Cadmium Accumulation in Rice. *J. Exp. Bot.* **2009**, *60*, 2677–2688. [CrossRef]
58. Hassan, M.; Zhang, G.; Wu, F.; Cheng, H.; Chen, Z.-H. Zinc Alleviates Growth Inhibition and Oxidative Stress Caused by Cadmium in Rice. *J. Plant Nutr. Soil Sci.* **2005**, *168*, 255–261. [CrossRef]

59. Saif, U.; Sarwar, N.; Bibi, S.; Ahmad, M.; Ok, Y.S. Effectiveness of Zinc Application to Minimize Cadmium Toxicity and Accumulation in Wheat (*Triticum aestivum* L.). *Environ. Earth Sci.* **2013**, *71*, 1663–1672.
60. Qaswar, M.; Hussain, S.; Rengel, Z. Zinc Fertilisation Increases Grain Zinc and Reduces Grain Lead and Cadmium Concentrations More in Zinc-Biofortified than Standard Wheat Cultivar. *Sci. Total Environ.* **2017**, *605–606*, 454–460. [CrossRef]
61. Rizwan, M.; Ali, S.; Hussain, A.; Ali, Q.; Shakoor, M.B.; Zia-ur-Rehman, M.; Farid, M.; Asma, M. Effect of Zinc-Lysine on Growth, Yield and Cadmium Uptake in Wheat (*Triticum aestivum* L.) and Health Risk Assessment. *Chemosphere* **2017**, *187*, 35–42. [CrossRef] [PubMed]
62. Palmer, C.M.; Guerinot, M.L. Facing the Challenges of Cu, Fe and Zn Homeostasis in Plants. *Nat. Chem. Biol.* **2009**, *5*, 333–340. [CrossRef] [PubMed]
63. Hänsch, R.; Mendel, R.R. Physiological Functions of Mineral Micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Curr. Opin. Plant Biol.* **2009**, *12*, 259–266. [CrossRef] [PubMed]
64. El Rasafi, T.; Oukarroum, A.; Haddioui, A.; Song, H.; Kwon, E.E.; Bolan, N.; Tack, F.M.G.; Sebastian, A.; Prasad, M.N.V.; Rinklebe, J. Cadmium Stress in Plants: A Critical Review of the Effects, Mechanisms, and Tolerance Strategies. *Crit. Rev. Environ. Sci. Technol.* **2020**, *52*, 675–726. [CrossRef]
65. Lozano-Rodriguez, E.; Hernàndez, L.E.; Bonay, P.; Carpena-Ruiz, R.O. Distribution of Cadmium in Shoot and Root Tissues. *J. Exp. Bot.* **1997**, *48*, 123–128. [CrossRef]
66. Astolfi, S.; Ortolani, M.R.; Catarcione, G.; Paolacci, A.R.; Cesco, S.; Pinton, R.; Ciaffi, M. Cadmium Exposure Affects Iron Acquisition in Barley (*Hordeum vulgare*) Seedlings. *Physiol. Plant.* **2014**, *152*, 646–659. [CrossRef]
67. Dong, J.; Wu, F.; Zhang, G. Influence of Cadmium on Antioxidant Capacity and Four Microelement Concentrations in Tomato Seedlings (*Lycopersicon esculentum*). *Chemosphere* **2006**, *64*, 1659–1666. [CrossRef]
68. Clemens, S.; Palmgren, M.G.; Krämer, U. A Long Way Ahead: Understanding and Engineering Plant Metal Accumulation. *Trends Plant Sci.* **2002**, *7*, 309–315. [CrossRef]
69. Yoshimoto, N.; Inoue, E.; Saito, K.; Yamaya, T.; Takahashi, H. Phloem-Localizing Sulfate Transporter, Sultr1;3, Mediates Re-Distribution of Sulfur from Source to Sink Organs in Arabidopsis. *Plant Physiol.* **2003**, *131*, 1511–1517. [CrossRef]
70. Gigolashvili, T.; Kopriva, S. Transporters in Plant Sulfur Metabolism. *Front. Plant Sci.* **2014**, *5*, 442. [CrossRef]
71. Astolfi, S.; Zuchi, S.; Passera, C. Role of Sulphur Availability on Cadmium-Induced Changes of Nitrogen and Sulphur Metabolism in Maize (*Zea mays* L.) Leaves. *J. Plant Physiol.* **2004**, *161*, 795–802. [CrossRef] [PubMed]
72. Wu, Z.; Naveed, S.; Zhang, C.; Ge, Y. Adequate Supply of Sulfur Simultaneously Enhances Iron Uptake and Reduces Cadmium Accumulation in Rice Grown in Hydroponic Culture. *Environ. Pollut.* **2020**, *262*, 114327. [CrossRef] [PubMed]
73. El-Ramady, H.; Prokisch, J.; Sári, D.; Singh, A.; Ghazaryan, K.; Rajput, V.D.; Brevik, E.C. Nanotechnology in the soil system: An ecological approach towards sustainable management. *Appl. Soil Ecol.* **2024**, *204*, 105669. [CrossRef]