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Iron-based nanomaterials reduce cadmium toxicity in rice (*Oryza sativa* L.) by modulating phytohormones, phytochelatin, cadmium transport genes and iron plaque formation

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Abstract

Rice is known to accumulate cadmium (Cd) in its grains, causing a severe threat to billions of people worldwide. The possible phytotoxicity and mechanism of 50-200 mg/L hydroxyapatite NPs (nHA), iron oxide NPs (nFe_2O_3) or nano zero valent iron (nZVI) co-exposed with Cd (100 μM) in rice seedlings were investigated. Three types of nanoparticles significantly reduced the bioaccumulation of Cd in rice shoots by 16-63%, with nZVI showing the greatest effect, followed by nHA and nFe_2O_3. A decrease in Cd content in the roots was observed only in the nZVI treatment, with values ranging from 8-19%. Correspondingly, nZVI showed the best results in promoting plant growth, increasing rice plant height, shoot and root biomass by 13%, 29% and 42%. *In vitro* studies showed that nZVI reduced the content of Cd in the solution by 20-52% through adsorption, which might have contributed to the immobilization of Cd in root. Importantly, the nZVI treatment resulted in 267% more iron plaques on the root surface, which acted as a barrier to hinder the entry of Cd. Moreover, all three nanoparticles significantly reduced the oxidative stress induced by Cd by regulating phytohormones, phytochelatin, inorganic homeostasis and the expression of genes associated with Cd uptake and transport. Overall, this study elucidates for the first time the multiple complementing mechanisms for some nanoparticles to reduce Cd uptake and transport in rice and provides theoretical basis for applying nanoparticles for reducing Cd accumulation in edible plants.

Keywords: Nanoparticles; Cadmium; Phytohormones; Antioxidant system; Iron plaque

Abbreviations

Abbreviation	Full name
NPs	Nanoparticles
TEM	Transmission Electron Microscope
CAS	Chinese Academy of Sciences
DI	Deionized water
ELISA	Enzyme linked immunosorbent assay
ZR	Zeatin riboside
DH-ZR	Dihydrozeatin riboside
IPA	Isopentenyl adenosine
BR	Brassinolide
IAA	Indole-3-acetic acid
GA3	Gibberellic acid 3
GA4	Gibberellic acid 4
JA-ME	Methyl jasmonate
ABA	Abscisic acid
SOD	Superoxide dismutase
POD	Peroxide dismutase
MDA	Malondialdehyde
CAT	Catalase
GSH	Glutathione
GSH-PX	Glutathione peroxidase
PCs	Phytochelatins
NPTH	Non-protein thiols

1 Introduction

Human health risks caused by heavy metals have attracted attention worldwide (Lu et al., 2015). Cadmium (Cd) is a toxic heavy metal commonly found in agricultural soils (Rehman et al., 2018). The World Health Organization (WHO) designated Cd and Cd-containing compounds as group I carcinogens due to their high toxicity and bioaccumulation potential (International Agency for Research on Cancer, 2012). Cd pollution in some countries such as China is particularly severe. The risk threshold of Cd in China is 0.3 mg/ kg, while the over standard rate of Cd pollution in agricultural soils is 7.0%, which is the highest inorganic pollutant (The Ministry Of Environmental Protection and The Ministry Of Land Resources, 2014; Zhao et al., 2015). Cd in soil displays a long decomposition cycle, high mobility and toxicity, resulting in reduced seed germination, plant growth inhibition and yield reduction (Kaya et al., 2019; Kaya et al., 2020; Rossi et al., 2018). Moreover, phytoaccumulation of Cd in the food chain may endanger human health (Zhou et al., 2022). Therefore, there is an urgent need of innovative and sustainable strategies to control the absorption of cadmium in edible crops.

Nanotechnology is the convergence of various sciences to provide disruptive technologies for manipulating nano at the atomic scale, creating new structures and applying nanomaterials (Poole Jr and Owens, 2003). Among them, nanoparticles (NPs) have been widely used in recent decades due to their unique chemical and physical properties (Ali et al., 2021; Mansoor et al., 2022; Zhao et al., 2022). Compared with traditional materials, NPs exhibit greater adsorption capacity, and catalytic and magnetic properties (Adeel et al., 2021; Shakoor et al., 2022). A recent report has revealed that the interaction between Cd and NPs and may alter the absorption, transfer and accumulation of Cd in rice (Zhou et al., 2021). Ji et al., (2017) reported that 100 mg/L of titanium dioxide alleviated the phytotoxicity of Cd, mainly because Cd was adsorbed by TiO_2. Similarly, (Wang et al., 2019) demonstrated that CeO_2 NPs alleviated the Cd stress and mitigated DNA damage in rice.

Cd is non-essential and no specific transporter proteins were identified for Cd uptake by rice (Tang et al., 2017). Instead, Cd uptake and transfer is mainly through the iron transporter, which involves *NRAMP* and *IRT* family transporters (Yang et al., 2014). Therefore, the competition between Cd and Fe absorb may cause an imbalance of Fe homeostasis in rice, leading to chlorosis of leaves (Zhang et al., 2020). Improving Fe homeostasis in plants using iron-based NPs thus potentially can help to relieve Cd phytotoxicity (Rizwan et al., 2016). In addition, the strong

adsorption of NPs for Cd may effectively reduce the bioavailability of Cd in the environment (Anjum et al., 2019). Some NPs made of nutrient element (e.g., Fe in Fe-based NPs and Ca, P in hydroxyapatite) may dissolve and release these nutrients thereby enhancing the tolerance of plants to the Cd induced toxicity. Fe-based NPs could also reduce Cd uptake by regulating Fe uptake transport-related proteins due to their shared transporters. Moreover, Fe^{2+} produced by the dissolution of iron-based NPs can contribute to the formation of iron plaques in rice roots, which can effectively prevent the uptake of Cd by plant (Sebastian and Prasad, 2016).

Three types of NPs including two Fe-based NPs (nano zero-valent iron (nZVI) and ferric oxide (nFe_2O_3)) and one natural inorganic mineral NPs (hydroxyapatite, nHA, $Ca_{10}(PO_4)_6(OH)_2$) were chosen to evaluate their impact on Cd accumulation in rice tissues due to their known beneficial effects on plants. For example, a whole life cycle culture study showed that Fe_2O_3 NPs reduced the harm of Cd to wheat growth and yield (Adrees et al., 2020a). Due to its high elemental P, nHA resulted in a 16.5 and 8-fold increase in aboveground and root fresh biomass of sunflower grown in phosphorus-deficient soils (Xiong et al., 2018). In addition, soil application of nHA promoted potato growth and reduced the Cd content in tubers by 17% compared to Cd treatment alone (Liu et al., 2018). All three NPs exhibited excellent biocompatibility at environment-relevant concentrations and were inexpensive, making them promising tools for Cd-contaminated soil. However, comprehensive studies concerning the physiological and biochemical effects of NPs and Cd on rice are still lacking.

In this study, we hypothesized that the addition of NPs could alter the uptake, translocation, and subsequent effects of Cd on rice, the most important food crop in many Asian countries (Zhang et al., 2021). Specifically, we investigated the effects of different NPs on rice growth under Cd stress by measuring a range of phenotypic and physiological parameters, including the biomass, root morphology, photosynthetic system, and inorganic nutrients. The stress status and plant tolerance to stress were also assessed by measuring the response of antioxidant systems and phytohormones in plants. In addition, the adsorption capacity of different NPs for Cd, the blockage of Cd by iron plaques and the expression of Cd transport genes were determined and their correlations with the observed biological effects were investigated.

2. Materials and methods

2.1 Characterization of NPs

Fe_2O_3 NPs, nZVI and nHA were all obtained at Pantian Co., Shanghai. The morphologies and primary sizes of all NPs were characterized using TEM (Tecnai F20, FEI, USA). The zeta potential and hydrodynamic sizes of NPs in Kimura B nutrient solution were analyzed with a Zetasizer Nano (ZS90, Malvern, UK). Detailed characterization of the NPs is shown in **Figures S1 and S2**.

2.2 Sorption of cadmium by NPs

Briefly, NPs powder were added to a glass vial containing Cd solution and subjected to ultrasonic treatment to disperse the NPs. After 14 days of incubation in the same environment as the plant culture, 10 ml of suspension was taken from the vial for the Cd determination. The detailed experiments are shown in Section 1, S1.

2.3 Plant culture and NPs treatment

Seeds (Y Liangyou 900) of rice (*Oryza sativa* L.) were acquired from the CAS. Rice seeds were disinfected with 10% H_2O_2 for 20 min and flushed completely using DI water. The seeds were placed evenly on a plastic tray containing a layer of gauze, then covered with a sheet of filter paper and immersed in 15 ml of DI water. The tray was then sealed with a film tray to avoid water loss. And germinate in a climatic incubator under darkness for 7 days (25°C). Similarly sized seedlings were anchored in sponge and planted into a plastic bottle with 250 ml ¼ strength Kimura B. Seedlings were cultivated in a climatic incubator (25/22 °C and a 16 h photoperiod). After 7 days of acclimation, rice seedlings were individually exposed to 50 and 200 mg/L nHA, nFe_2O_3 or nZVI for 14 days or 100 μM Cd + NPs. Seedlings treated with 100 μM Cd without addition of NPs were used as a control. Seedlings without any treatment were used as blank control. The Kimura B was added regularly to an initial volume of 250 ml every other day, followed by the determination of Cd levels in the hydroponic system. At day 14, all seedlings were harvested and rinsed thoroughly with 10% of nitric acid and DI water to remove the NPs and Cd attached on root

surface. After length and biomass measurements, fresh roots and leaves were stored at
-80°C for subsequent analysis.

2.4 Root morphology and iron plaque extraction

An automated scanner (Epson expression 1680, Japan) was used to scan the rice roots, followed by analysis of root parameters using RHIZO software (Pro 2007d, Quebec, Canada). The extraction of iron plaque on the root surface was performed by the citric acid bicarbonate dithionate (DCB) method as described by Liu et al., (Liu et al., 2006). Briefly, fresh roots were soaked in 20 ml of 125 mM $NaHCO_3$ and 30 mM $Na_3C_6H_5O_7$ mixture, the added 0.4 g $Na_2S_2O_4$ powder. The mixture containing roots is stirred and placed at 25 °C for 1 hour. The roots were rinsed with DI water, and all eluents were collected and diluted to 50 ml with DI water, followed by Fe and Cd measurements. The digestion and determination methods of elements (Cd and other mineral elements) are shown in **Section 1, S2**.

2.5 Determination of antioxidative system, metal chelators, chlorophyll content and phytohormones

The phytochelatin (PCs), glutathione (GSH), non-protein thiol (NPTH) that can bind Cd^{2+} were measured by testing kit. MDA content and the activities of CAT, SOD, POD and GSH-PX were determined using testing kit. Spectrophotometric methods were used to determine chlorophyll in leaves. ELISA was used to quantify the level of nine phytohormones in the roots, according to the previously described methods (Ma et al., 2020). Detailed information is shown in **Section 1, S3, S4 and S5**.

2.6 Extraction of subcellular Cd fractions

Cells were isolated in three fractions three fractions using centrifugation according to the method of Wan et al., (Wan et al., 2019). Briefly, the tissue samples were homogenized in buffer and centrifuged at different speeds to collect three cell fractions. These three parts were digested with nitric acid and determined by ICP-MS. Details are presented in **Section1, S6**.

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2.7 RT-qPCR Analysis of Cd Transporters

Total RNA extraction, complementary DNA synthesis and RT-qPCR were conducted according to the method of Ma et al., (Ma et al., 2021). The relative expression of four genes related to Cd absorption and transportation, including *IRT1*, *Nramp5*, *HMA3* and *LCT1*, was computed by a $2^{-\Delta\Delta Ct}$ method. Details are presented in **Section 1, S8**.

2.8 Data analysis

SPSS 20.0 was used to conduct the data analysis. Statistical significance was executed through one-way ANOVA. The average values of each treatment were compared using the Tukey's HSD at $p < 0.05$ confidence level.

3 Results

3.1 Different impacts of nanoparticles on rice phenotypes under cadmium exposure

All rice seedlings survived after 14 days of exposure (**Figure 1**). Inhibitive effect was observed only for 200 mg/L of nHA treatment which slightly reduced the root fresh weight by 7% (**Figure 1D**). nZVI showed stronger effects than other NPs both at low and high concentrations, and 200 mg/L of nZVI increased the plant height, shoot weight and root weight by 13%, 29% and 42%, respectively. Cd (100 μM) significantly inhibited the rice seedling growth. Cd treatment reduced the shoot and root lengths, shoot and root biomass by 45%, 23%, 67% and 30%, respectively (**Figure 1**). The presence of NPs mitigated the phytotoxicity of Cd and improved rice growth parameters (e.g., biomass and root morphology) in a dose-dependent manner (**Figure 1E, F and S3**). Specifically, all three NPs increased the fresh shoot and root biomass by 58.27%-106.51% and 13.32%-66.52%, respectively, compared to the Cd treatment (**Figure 1C, D**). Again, nZVI at 200 mg/L showed the strongest positive effects on alleviating the Cd toxicity, resulting in 35%, 17%, 107% and 67% increase in plant height, root length, shoot biomass and root biomass, correspondingly. Furthermore, nHA and nZVI at 200 mg/L also improved all the root parameters

187   (**Figure S4**). In particular, nZVI at 200mg/L enhanced the total root length, average

188   root diameter, total surface area and total root volume by 135, 82, 127 and 163%,

189   respectively.

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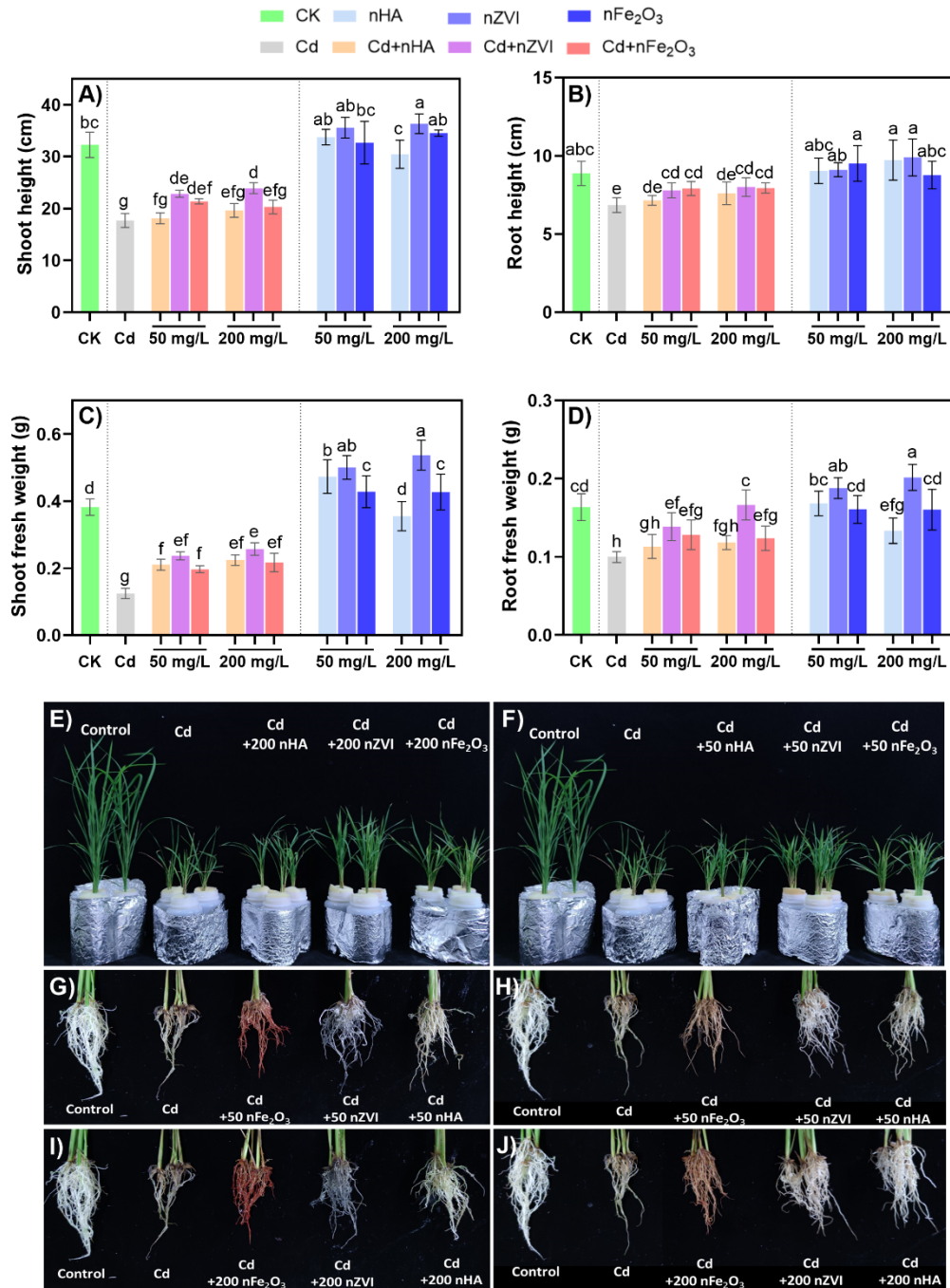
Figure 1. Length (**A** and **B**) and fresh biomass (**C** and **D**) of rice seedlings cultivated

to Cd, NPs or Cd + NPs for 2 weeks. Values indicate the mean ± SD of 6 replicates.

Significant differences at $p < 0.05$ are indicated by different lowercase letters.

Representative physiological pictures of rice exposed to Cd and NPs. (**E** and **F**)

represent rice images as affected by 50 and 200 mg/L NPs for 14 days, respectively. Root images of rice treated with Cd and NPs. (**G** and **I**) taken before cleaning NPs on the root surface. (**H** and **J**) taken after cleaning NPs on the root surface.

3.2 Nanoparticles regulate photosynthesis, antioxidative system and metal chelators

Compared with Cd treatment, the addition of NPs enhanced the leaf moisture (77%-127%), Cha (52%-131%), Chb (109%-295%) and SPAD values (39%-82%). High concentration of nZVI (200mg/L) showed the strongest positive effects, resulting in an increase of 127%, 131%, 295% and 82% in leaf moisture, Cha, Chb and SPAD values, respectively (**Figure S5**). MDA contents in shoots with Cd exposure alone was significantly increased 48% relative to the control, which means Cd exposure caused lipid peroxidation (**Figure S6A**). Cd stress activated the plant antioxidant system, elevating POD, SOD and GSH-PX activities by 99%, 108% and 25%, respectively. Notably, the addition of NPs further elevated POD and SOD activities by 169%-308% and 91%-209%, and reduced the MDA contents (65%-76%). Additional discussions about photosynthesis and enzymatic antioxidant system are provided in the **Section1, S9 and S10**. These results suggest that NPs treatments enhanced the enzymatic antioxidant systems. Under Cd stress, the non-enzymatic antioxidant system such as GSH and non-protein thiols (NPTH) were not significantly altered by NPs supplement (**Figure 2A** and **2B**), except for ZVI alone at 200 mg/L enhanced NPTH level (98%). Previous evidence has suggested that phytochelatins (PCs) play important roles in the detoxification of Cd in plants (Guha et al., 2020). Interestingly, PCs in rice increased by 208-346% as compared with the control after exposure to high concentrations of NPs alone (**Figure 2C**). The same trend in PCs was also observed for Cd + NPs co-exposure, with nZVI showing the strongest effects. Specifically, nZVI at 200 mg/L elevated the content of NPTH and PCs by 88% and 49% compared to the Cd treatment (**Figure 2B and 2C**). nFe_2O_3 also significantly elevated PC content (73%-84%), but it did not significantly elevate NTPH (24%-46%). The above results indicate that the addition of NPs can modulates the antioxidant system and PCs levels in plants, which protects plant from Cd stress.
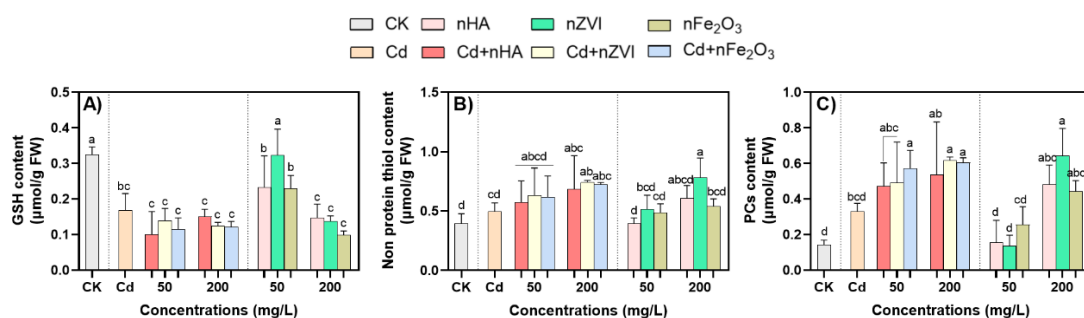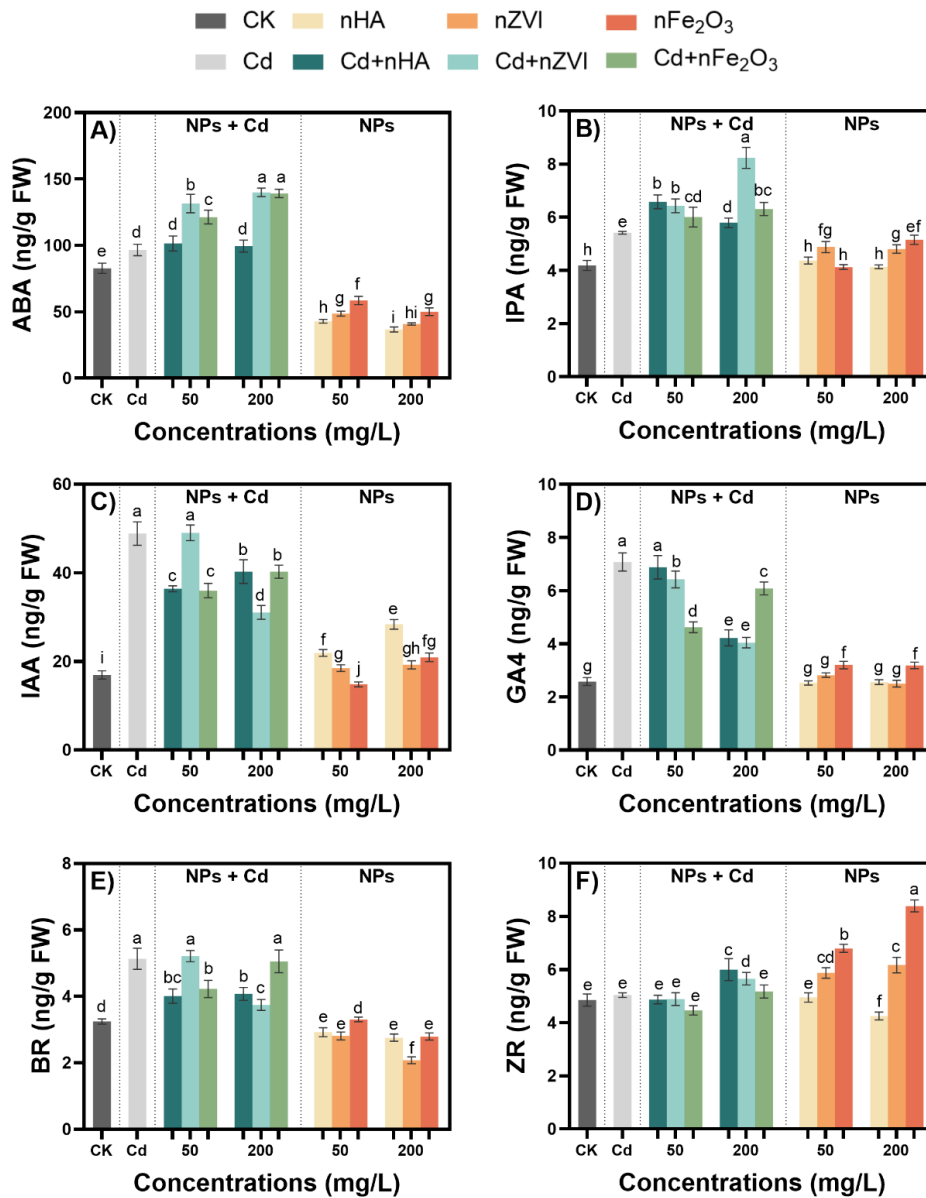
Figure 2. The content of GSH (A), Non protein thiol (B) and phytochelatin (C) in rice shoots exposed to Cd, NPs or Cd + NPs for 2 weeks. Values indicate the mean ± SD of 4 replicates. Significant differences at $p < 0.05$ are indicated by different lowercase letters.

3.3 Regulation of phytohormones by nanoparticles

Under Cd stress, all phytohormones except for ZR were significantly up-regulated compared to the control (**Figure 3**). The phytohormones displayed similar trends for NPs alone and Cd+NPs except for ABA and ZR. The ABA content significantly decreased by 29-55% when exposed to NPs alone, indicating that the exposure to NPs alone did not cause a significant stress response. A previous report demonstrated that excess ABA was associated with plant growth inhibition (Brookbank et al., 2021). However, iron-based NPs resulted in a significant 20-72% increase of ZR, which is considered an antagonist of ABA, and our results are consistent with this trend (Zhang et al., 2021). Overall, a more significant up-regulation of ABA and IPA occurred in the case of Cd + NPs. The addition of nZVI and nFe_2O_3 significantly up-regulated ABA by 36%-45% and 25%-44% compared to the Cd control (**Figure 3A**). Similarly, nZVI and nFe_2O_3 at 200 mg/L resulted in a significant increase in IPA by 52% and 16% (**Figure 3B**), consistent with the physiological results. In contrast, a decreasing trend was observed for IAA, GA4 and BR after NPs exposure (**Figure 3C-3E**). Once again, nZVI at 200 mg/L showed the strongest effect, indicating its greatest effectiveness in mitigating Cd stress among the three NPs investigated in this study. For IAA, the addition of 200 mg/L nHA, nZVI and nFe_2O_3 decreased IAA by 17%, 36% and 17%, respectively (**Figure 3C**). Overall, our results suggest that some NPs can mitigate Cd toxicity, at least in part, by

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249   regulating defense-related phytohormone levels.

Figure 3. Phytohormone contents including ABA, IPA, IAA, GA4, BR and ZR in rice
roots exposed to Cd, NPs or Cd + NPs for 2 weeks. Values indicate the mean ± SD of
4 replicates. Significant differences at $p < 0.05$ are indicated by different lowercase
letters.

3.4 Homeostasis of inorganic mineral nutrients

The inorganic homeostasis was severely imbalanced in rice under Cd stress, resulting
in significant alterations in three elements (Mg, K and Zn) in roots and five elements
(Mg, Ca, Cu, Zn and P) in shoots (**Figure 4**). High concentrations of nZVI and

nFe$_2$O$_3$ resulted in 131% and 255% increases of Fe in roots (**Figure 4A**). Similarly, 200 mg/L of nHA resulted in 76% and 31% increase in Ca and P in the root. However, the increase of P was not significant (29%-31%), probably due to the high content of P in the nutrient solution. Notably, both nZVI and Fe$_2$O$_3$ NPs at 200 mg/L resulted in a 51% increase in Cu content in the roots, with no effect on other inorganic elements except for Fe. Inorganic nutrient homeostasis in shoots was more sensitive to NPs (**Figure 4B**) than roots. In the treatment of Fe-based NPs, 200 mg/L nZVI resulted in 58% and 24% increases in Cu and Zn elements. At 200 mg/L, nHA reduced the content of all five elements in the leaves, i.e., Mg, Ca, Fe, Cu, and Zn by 25%, 37%, 65%, 61%, and 28%, respectively. The results suggested that the addition of nHA might have blocked the *in-planta* transport of inorganic nutrients from underground to aboveground.
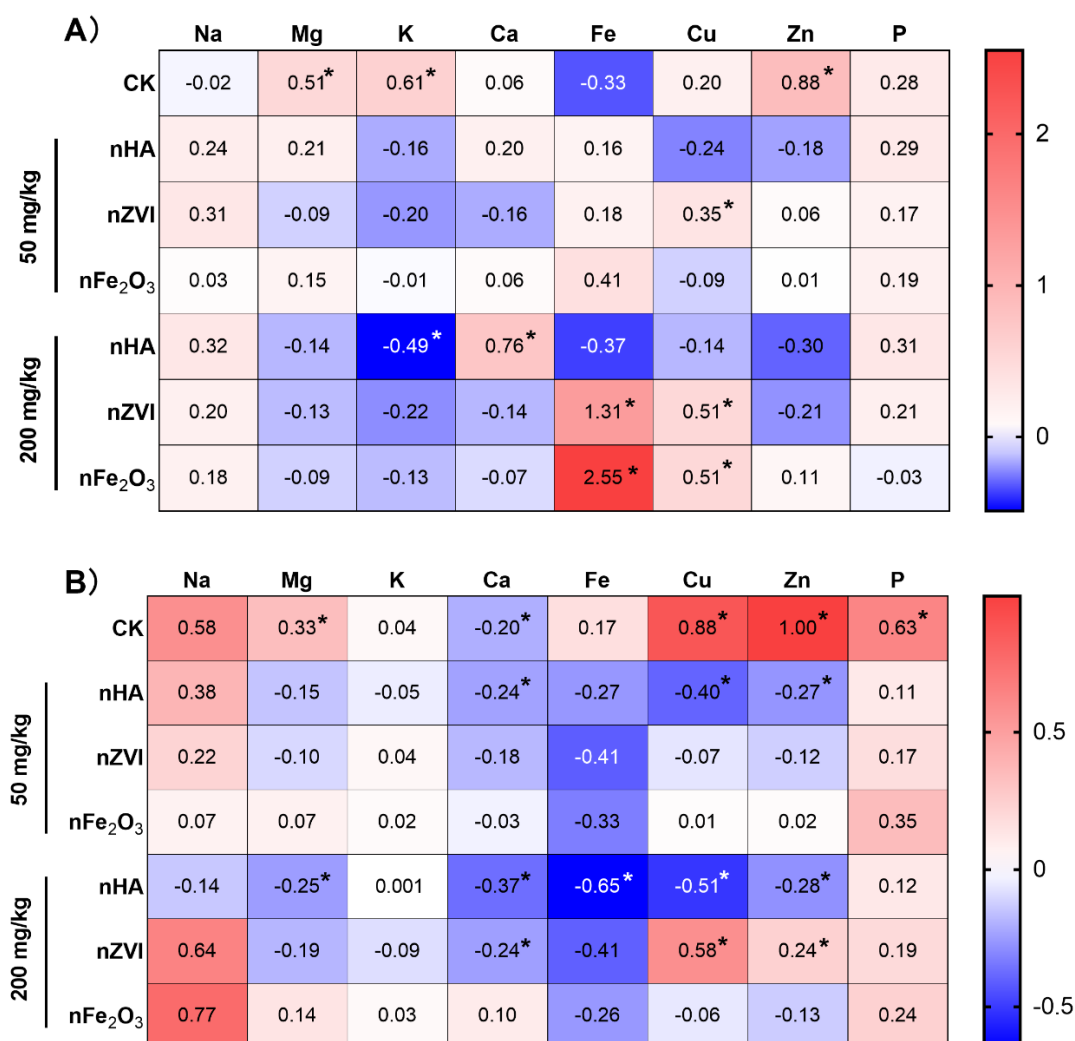
272 **Figure 4.** Thermogram illustrating the homeostasis of inorganic nutrients in rice root

273 (A) and shoot B) modulated by Cd and Cd + NPs after two weeks. Numbers represent

274 the multiplicity of change in content of elements compared to the Cd alone treatment.

275 Negative values denote a decrease in content, while positive values are the opposite.

276 Values indicate the mean ± SD of 6 replicates. Significant differences compared with

277 the Cd alone control at $p < 0.05$ (n=4) are indicated by *.

278 *3.5 Total cadmium and subcellular distribution in rice tissues*

279   The absorb and subcellular distribution of Cd in plant were further investigated

280 to explore the link between Cd accumulation and plant physiological regulators. Cd

281 accumulation in the roots of plants treated with Cd+NPs was not significantly reduced

282 except for the 200 mg/L nZVI treatment (**Figure 5A**). However, Cd accumulation in

283 the shoots was more significantly reduced after NPs exposure (**Figure 5B**).

284 Specifically, rice exposed to 200 mg/L nHA and nZVI resulted in a 75% and 80%

285 reduction in Cd in shoots. The effects of different NPs on Cd accumulation of shoots

286 upon exposure to Cd are consistent with the results of growth differences in **Figure 1**.

287 Further investigation of the mechanisms related to the effect of NPs on the absorb and

288 translocation of Cd was conducted by measuring the Cd subcellular distribution. As

289 shown in **Figure 5C-D**, the majority of Cd was in the soluble fraction (F3, 42–74%),

290 followed by the cell wall fraction (F1, 17–48%) and organelle fraction (F2, 4–13%),

291 both for shoots and roots. The soluble fraction consists mainly of vesicles, which are

292 the main location to preferentially bind Cd to minimize its damage to the cell wall or

293 entry into the organelle (Wang et al., 2009). Compared with Cd alone treatment, nZVI

294 at 200 mg/L significantly enhanced the percentage of Cd in soluble fractions of roots

295 by 20% and decreased the cell wall and organelles fractions by 8% and 12%,

296 respectively (**Figure 5C**). A similar phenomenon was observed in shoots (**Figure 5D**),

297 although the difference was not significant.
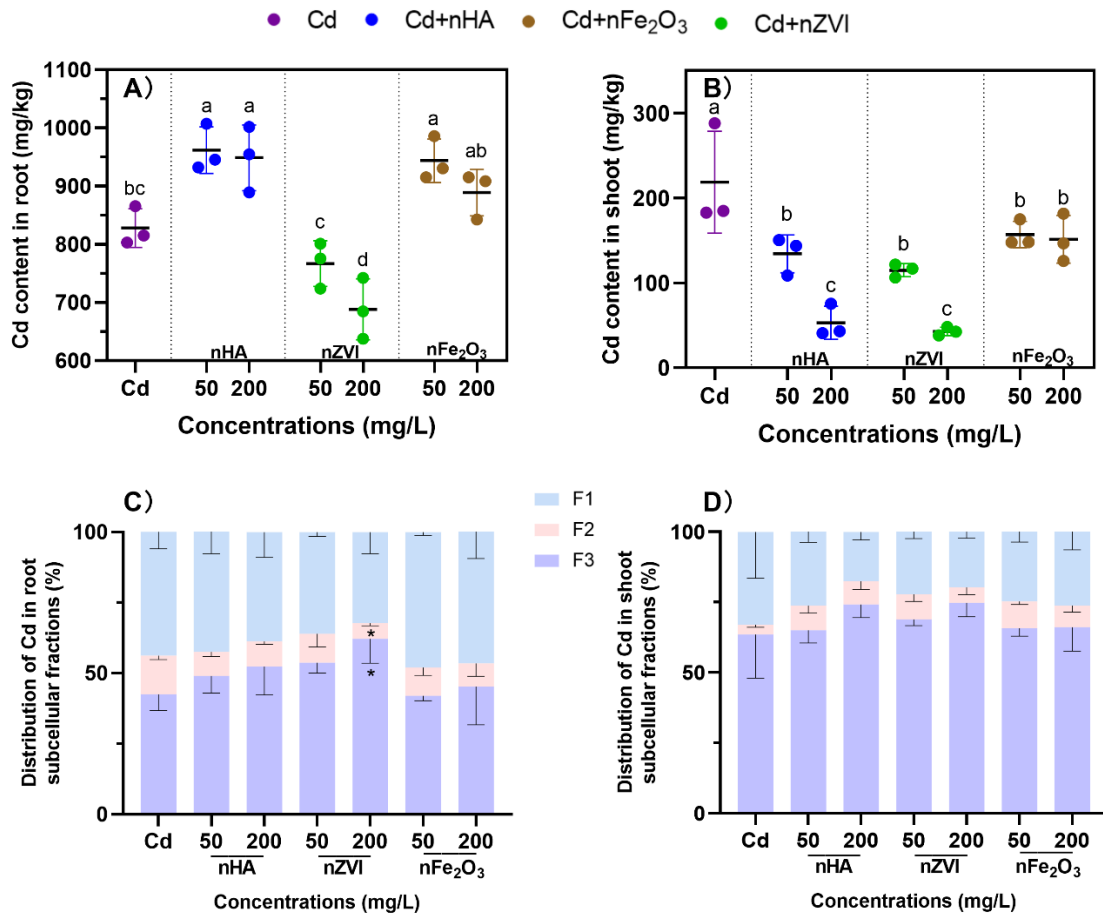
Figure 5. Total Cd content in rice seedlings (A-B). Cd in root (A), and shoot (B) after 14 days of exposure to Cd + NPs. Cd distribution of subcellular components in root (C) and shoot (D). F1, F2 and F3 refer to the cell wall, organelle and soluble fractions. Values indicate the mean ± SD of 3 replicates. Significant differences at $p < 0.05$ are indicated by * or different lowercase letters.

3.6 Cadmium sorption in solution and cadmium content in iron plaque

Dynamic chemical changes between Cd and NPs in the nutrient solution may be another important factor affecting Cd uptake by plants. The findings indicated that the sorption capacity of nFe₂O₃ for Cd was much less than that of nHA and nZVI, and only 3-8% of the initial Cd content in the nutrient solution was adsorbed on nFe₂O₃ (**Figure 6A**). However, nHA and nZVI significantly reduced the Cd content in the nutrient solution by 12%-24% and 20%-52%, respectively. The zeta potential of NPs in nutrient solution is critical to the adsorption of NPs. Our data showed that the zero charge points (nHA, nZVI and nFe₂O₃) of the three NPs were 4.4, 4.9 and 7.3,

respectively (**Figure S2**). Therefore, at the beginning of Cd exposure, Cd^{2+} will strongly adsorb to the negatively charged nHA and nZVI through electrostatic interactions. However, nFe_2O_3 has a zeta potential of about +14.5 mV at pH 5.5, and the electrostatic repulsion prevents the approach of Cd and further interactions. Therefore, the adsorption capacity of nFe_2O_3 for Cd is much lower than the other two NPs. In addition to Cd adsorption via NPs, Cd uptake by plants is a primary process leading to the reduction of Cd in nutrient solutions (**Figure 6B-D**). The uptake of Cd by plants was around 28% of its initial concentration in the Kimura B containing only Cd. With the addition of 200 mg/L nZVI, the plant uptake of Cd only accounted for 6% of its initial content. The remaining 46% and 48% of Cd were either adsorbed by nZVI or retained in the nutrient solution, respectively, emphasizing the essential role of nZVI in reducing the Cd bioavailability (**Figure 6C**).
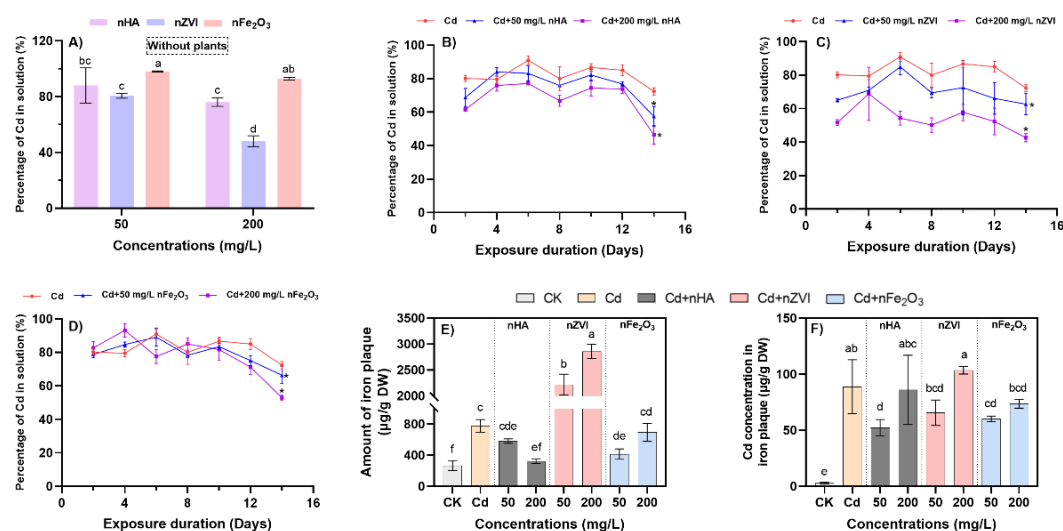
Figure 6. The residual percentage of cadmium in nutrient solution after 14 days of exposure without plants (A). Curves of Cd concentration in the nutrient solution during the exposure with plants (B-D). (B) nHA; (C) nZVI; (D) nFe_2O_3. The amount of iron plaque (E) on the root surfaces and cadmium content in iron plaque (F) after 14 days exposure. Values indicate the mean ± SD of 4 replicates. Significant differences at $p < 0.05$ are indicated by * or different lowercase letters.

In order to accommodate the flooded conditions, rice can deliver O_2 from the shoot to the inter-root environment, causing the oxidation of Fe^{2+} and Mn^{2+} to form a

reddish-brown iron oxide film (Iron plaque) (Amaral et al., 2017). The content of iron plaque and the Cd in the plaques were determined to explore the effect of NPs on iron plaque formation and how iron plaques influence the absorption of Cd by roots. The total iron plaque amount was significantly increased by 190% in the Cd exposure alone compared to the blank control. At 200 mg/L nHA, iron plaques were markedly decreased by 58% compared to the Cd control (**Figure 6E**). Further analysis indicated that the total iron plaque produced on the root surface was positive correlated with the Cd content in the nutrient solution in the Kimura B for the non-Fe-based NPs added system (R^2 = 0.786, **Figure S9**). For the system with Fe-based NPs addition, the total amount of iron plaques was increased with increasing dose of Fe-based NPs. In particular, nZVI significantly increased the mass of iron plaques by 185% and 267% relative to the Cd control, which was in agreement to the results for the Cd content in roots. In the presence of 50 mg/L nHA and nFe_2O_3, Cd levels in iron plaques were significantly reduced by 32-41% relative to the Cd-exposed system alone; the remaining treatments had no significant effect on Cd levels in iron plaques (**Figure 6F**). In addition, the Cd content in iron plaques was positively correlated with NPs concentration. These discoveries indicate that the rise in iron plaques might be a fundamental cause of the decline in Cd content in rice.

3.7 Relative expression of Cd transporters

The relative expression of Cd uptake and translocation-related proteins (*OsNRAMP5*, *OsHMA3*, *OsIRT1*, and *OsLCT1*) in rice shoots and roots were measured to explore the molecular mechanisms for altered Cd accumulation by NPs (**Figure 7**). *NRAMP5*, a resistance- related macrophage protein, is known to be the main transporter protein for Cd^{2+} and Fe^{2+} uptake in rice roots (Yang et al., 2014). Similarly, Cd could be taken up through the Fe (II) transporter, the Fe -regulated transporter 1 (*IRT1*) in rice roots (Lee and An, 2009). *OsHMA3* localized on the tonoplast is a member of the heavy metal ATPase (*HMA*) family, which could sequester Cd into vacuoles to reduce the transport of Cd to shoots (Lu et al., 2019). Interestingly, we found that the expression of *OsNRAMP5*, *OsHMA3* and *OsIRT1* genes showed a similar trend both in the roots and shoots. Expression of these three genes in the roots of Cd treated rice was upregulated about 500%, whereas co-exposure to nHA and nZVI with Cd restored their expression back to control levels. Surprisingly, co-exposure to nFe_2O_3 with Cd caused an approximately 800% increase in *OsNRAMP5*, *OsHMA3* and *OsIRT1* expression in rice roots (**Figure 7A, B, C**). To better understand the potential effects of NPs on Cd accumulation in the aboveground tissues, the expression of low-affinity cationic transport protein (*LCT1*) was determined. *LCT1* regulates the transport of Cd through the roots to the aboveground tissues (Uraguchi et al., 2011). An approximately 500% increase in expression was observed in roots in all treatments except for the $Cd+nFe_2O_3$ exposure (**Figure 7D**). However, nZVI and nFe_2O_3 up-regulated *LCT1* expression in shoots by approximately 500% and 1800%, respectively.
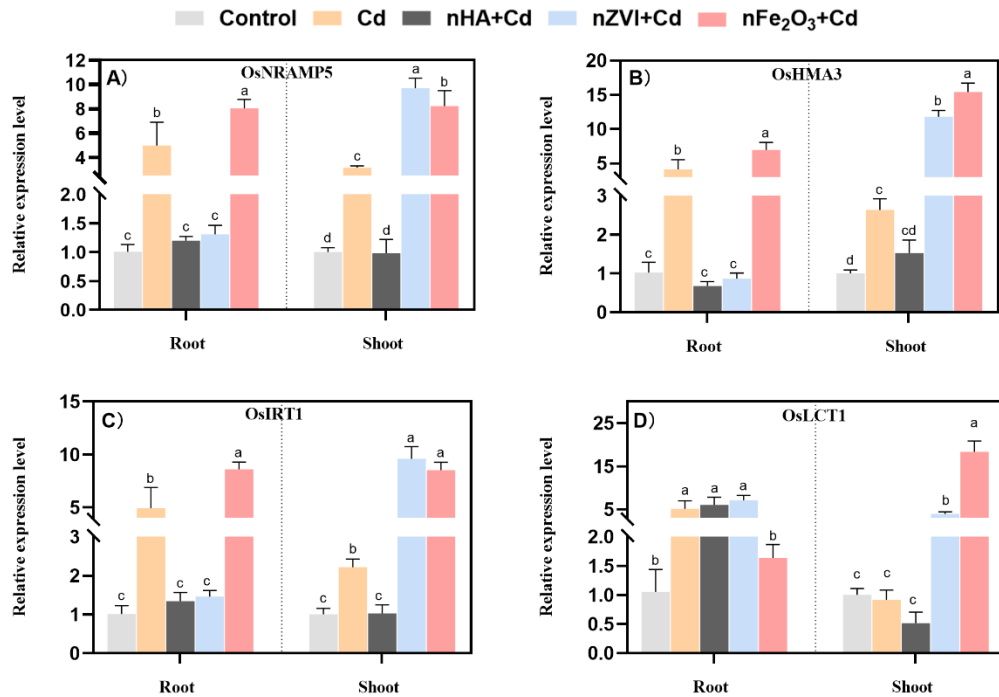
Figure 7. Relative expressions of genes involved in Cd assimilation and transport in rice root and shoot under Cd + 200mg/L NPs application. *NRAMP5* (A), *HMA3* (B), *IRT1*(C), *LCT1* (D). Values indicate the mean ± SD of 4 replicates. Significant differences at $p < 0.05$ are indicated by different lowercase letters.

4 Discussion

4.1 Regulation of plant physiological and biochemical systems by NPs

The NPs treatment alone either did not affect or increased the plant height and biomass except for nHA, whereas Cd treatments significantly inhibited rice growth. All different types of NPs reduced the phytotoxicity of Cd and improved the growth parameters of rice in a dose-dependent manner. Both at low or high concentrations of treatment, nZVI showed the best results for growth promotion. Similar results were also observed in one of our previous Fe-based NPs studies, where the "fertilizer effect" and "nano stimulus effect" of nZVI treatment resulted in a rise in chlorophyll, hormone regulation, and enhanced expression of iron transporter protein in rice (Li et al., 2021). To investigate the potential mechanisms of NPs to alleviate Cd stress, we conducted studies on both the physiological and biochemical systems of plants, the

392   formation of iron plaque and the adsorption behavior of NPs.

393   Cd is known to cause membrane lipid peroxidation and inhibit photosynthesis

394   (Ma et al., 2020; Rossi et al., 2017). Our results suggest that the enhanced biomass

395   and seedling elongation may be partly attributed to the enhanced photosynthesis by

396   NPs treatments. Compared with Cd treatment, the addition of NPs enhanced the leaf

397   moisture, chlorophyll content and antioxidative system. The positive effects of NPs

398   might be related to the release of mineral elements (Fe, P, Ca) which can support

399   photosynthesis and plant growth. Compared to conventional ion treatment, the slow-

400   release effect of nanoparticles can effectively avoid plant toxicity caused by a large

401   supply of ions in a short period of time (Cao et al., 2022). Iron is the central metal for

402   several vital enzymes (e.g., cytochromes) involved in electron transfer during

403   photosynthesis (Tagawa et al., 1963). Similar results were reported previously that

404   foliar application of 20 mg/L nFe_2O_3 increased chlorophyll a, b and carotenoid

405   concentrations by 70%, 139% and 119%, respectively (Hussain et al., 2019). Similarly,

406   P is a component of chloroplast bilayer membrane, grana and adenosine triphosphate

407   (ATP), which plays an essential role in photosynthesis energy conversion (Veneklaas

408   et al., 2012). The increase in photosynthetic pigments caused by the supply of mineral

409   elements indirectly led to an increase in photosynthetic rate, thus enabling rice to

410   accumulate more organic matter (Kaya et al., 2020).

411   Under the Cd stress, plants produce excessive harmful reactive oxygen species

412   (ROS), leading to an increase in MDA content, an important biomarker of lipid

413   peroxidation (Zhou et al., 2022). We found that the MDA content was relatively high

414   in rice shoots not treated with NPs due to heavy metal toxicity. The addition of all

415   types of NPs significantly increased the POD and SOD activity, followed by the

416   reduction of MDA contents, indicating that the addition of NPs could further trigger

417   the defense mechanism of antioxidant enzymes. In agreement with our study, nZVI

418   has been shown to increase SOD and POD in sunflower leaves, followed by the

419   decrease of MDA (Michálková et al., 2017). Adress et al., (2020b) also demonstrated

420   that the soil application of iron-based NPs promoted wheat growth and the SOD and

POD activities under Cd stress. Recent studies reported the specificity of NPs, not only as key substances for regulating ROS homeostasis, but also for directly scavenging excess ROS to enhance crop stress resistance (Altaf et al., 2022; Zhao et al., 2022). We also explored non-enzymatic antioxidant systems, but the differences after NPs treatment were not significant (NPTH), suggesting that enzymatic antioxidant systems are the main mechanism of action. GSH further decreased after treatment with NPs, demonstrating the involvement of GSH in the elimination of ROS and alleviation of oxidative stress (Kaya et al., 2021). In addition, the important role of PC in the detoxification of plants against Cd was clearly indicated in several reports (Guha et al., 2020; Kaya et al., 2022). PC could form complexes with toxic metal ions in cytoplasmic solution and subsequently translocate them to the vesicles (Yadav, 2010). A significant rise in PC was observed in the case of Cd + NPs co-exposure, with nZVI showing the strongest effect. Notably, the PC-induced rise in Cd content in the vesicles was consistent with that observed in the subcellular fractions, which provides strong evidence that NPs induced PC production is one of the important mechanisms for the mitigation of Cd stress. Overall, our results suggest that regulation of the enzymatic antioxidant system and PC production are important mechanisms for NPs-induced relief of Cd stress.

Phytohormones are crucial in plant growth and their regulation is an important mechanism for abiotic stress alleviation (Wani et al., 2016). The present study identified the important role of ABA and IPA in the mitigation of Cd stress. ABA is a 'stress phytohormone', which is typically increased when plants tried to cope with stresses (Vishwakarma et al., 2017). The supplementation of NPs under Cd stress led to a further increase of ABA indicating that NPs could enhance the Cd stress tolerance of rice. IPA is a plant growth regulator with similar biological activity as IAA but is defined as a cytokinin (Mano and Nemoto, 2012). Interestingly, the adding of NPs under Cd stress seemed to promote the synthesis of IPA. This might be attributed to the NPs promoted production of new rice roots which contain large number of cytokinin. It is important to point out that the contents of phytohormones in plants are

450 often interrelated. For instance, GA4 is usually positively correlated with the content

451 of IAA. The IAA content is also correlated with BR because IAA is regulated by BR

452 through the inhibition of the auxin oxidase activity. These connections among the

453 three phytohormones explained the similar trend observed in this study (Kandhol et

454 al., 2022; Tripathi et al., 2022; Wang et al., 2022). In addition to those reported in the

455 present study, some previous reports have also demonstrated that NPs can modulate

456 plant phytohormones to alleviate environmental stress, such as TiO_2 NPs alleviated

457 Cd stress (Ji et al., 2017) and CeO_2 NPs alleviated N overload or deficiency (Wang et

458 al., 2020). Undoubtedly, further studies are required to gain more insight into Cd and

459 NPs induced phytohormonal responses to optimize this potentially important abiotic

460 stress management strategy.

461   Cd stress leads to a serious imbalance in plant mineral element homeostasis,

462 which may be one of the important causes of stunting in rice. We found that the Cd

463 content in rice decreased after the addition of NPs, and correspondingly the mineral

464 homeostasis was altered. This may be partly due to the uptake of ions by plants in the

465 form of chemical equivalents, and the competing uptake between ions (Cui et al.,

466 2008; Qin et al., 2020). In addition, previous reports also showed that the small size of

467 NPs may affect ion transporters, thus changing the mineral homeostasis (Zhou et al.,

468 2021). Thus, the reduction of Cd induced by nZVI (**Figure 5B**) may have elevated the

469 concentrations of Cu and Zn in shoots. Interestingly, nHA displayed different effects

470 on nutrient homeostasis in plant tissues from Fe-based NPs. We found a further

471 reduction of the five mineral elements in the stems resulting from the addition of high

472 concentrations of nHA compared to the Cd treatment alone. The greater interference

473 of ion transport by nHA than nZVI might be an important reason why nHA was less

474 effective than nZVI in alleviating Cd stress. The growth and development of rice are

475 dependent on a variety of inorganic nutrients. Deficiency or excess of these elements

476 can even lead to poor development (Zhang et al., 2021). The results indicate that the

477 regulation of inorganic nutrient homeostasis may be another meaningful way for NPs

478 to alleviate Cd stress. High concentrations of nZVI significantly elevated the Fe and

Cu contents in the roots and Cu and Zn elements in the shoots to a comparable level in the control plants. Despite the rise of P after nHA addition, nHA might result in a further imbalance in the homeostasis of the remaining inorganic nutrients. Fe is an essential component of Fe oxygen reduction protein in plants, which is involved in plant photosynthesis, nitrate reduction, biological nitrogen fixation and other electron transfer (Hussain et al., 2019). Similarly, Cu plays a crucial role in electron transfer during photosynthesis, and it is as well an essential part of SOD (Ma et al., 2020). Therefore, the high Fe and Cu content induced by nZVI treatment may effectively promote photosynthesis, improve nutrient transport (Pishkar et al., 2022) and promote growth of rice under Cd stress.

4.2 Regulation of iron plaques and influence on Cd uptake and distribution by NPs

The results of Cd uptake and distribution showed that co-exposure of nZVI with Cd induced significantly lower Cd accumulation in the rice compared to the other two NPs. It should also be noted that all NPs except nZVI resulted in elevated Cd content in the roots, which may be due to the entrance of Cd adsorbed on NPs (This fraction of cadmium or other elements can be desorbed again in the plant). However, this fraction of Cd will be mostly retained in the roots because of the low upward transport of NPs, resulting in a relatively high Cd content in the root compared to the shoot. Gong et al. (2017) also demonstrated that nZVI at 1 μg/L promoted Cd accumulation by 50% in roots of ramie. The significant reduction of Cd in shoots when Cd and NPs were co-exposed suggests that the effect of NPs in mitigating phytotoxicity is a function of reduced Cd absorb and translocation. The Cd content in the root system was significantly reduced by nZVI and most of the residual Cd was translocated to the vesicular fraction. In addition, the high concentration of nZVI significantly enhanced the length and weight of the root system, indicating that it effectively relieved the Cd stress in rice roots. Interestingly, more severe Cd toxicity may significantly lower Cd in the soluble fraction in plant roots, resulting in more Cd accumulation in the cell walls than shoot (Lu et al., 2017). The constituents of plant cell walls could provide numerous potential ligands with different functional group, like hydroxyl, carboxyl,

508 aldehyde and amino groups (Wang et al., 2009), that can provide adsorption sites or

509 form complexes with Cd. Compared with Cd alone treatment, the relatively higher

510 integrity of the cell wall treated with nZVI reduced the number of Cd ions entering

511 the organelles, thus reducing the damage of Cd to the root cells. It should be noted

512 that the destructive method (Differential centrifugation method, detailed in

513 Supplementary materials S6) that used in this study may cause redistribution of Cd in

514 vacuole to cell walls during the sample preparation. However, this potential drawback

515 may not significantly change the overall observations in this study even though it may

516 underestimate the actual soluble fraction of Cd in the vacuoles.

517   The adsorption results further demonstrated that the final Cd concentration in the

518 Kimura B after 14 days of nZVI exposure was much lower than that of nHA and

519 nFe_2O_3, which can be attributed to the strong adsorption capacity of nZVI for Cd. The

520 surface charge of NPs and the solution pH are important factors affecting the uptake

521 of NPs. The adsorption capacity of nFe_2O_3 for Cd is much lower than that of the other

522 two NPs due to electrostatic repulsion (nFe_2O_3 has a zeta potential of about +14.5 mV

523 at pH 5.5) (Gao et al., 2004). Plants may release protons or alkaline secretion to

524 balance Cd uptake as a response to environmental stress, which would also alter

525 nutrient solution pH, Cd morphology, and NPs adsorption effects (Ryan et al., 2001).

526 In addition, the nanomaterials themselves also have the potential to alter the

527 environmental pH (Adeel et al., 2021; Shakoor et al., 2023). pH is a key factor

528 affecting the bioavailability of Cd, which usually shows a tendency to enhance

529 bioavailability with decreasing pH (Van Gestel and Koolhaas, 2004). High

530 concentration of nZVI induced a slight increase in the pH of rice solution compared to

531 the initial pH, while the opposite was observed for nFe_2O_3 and nHA treatments,

532 suggesting that nZVI can alleviate Cd stress by changing the pH. For a detailed

533 discussion of the dynamics of pH in solution see Section 1, S11.

534   Iron plaque function as a barrier to prevent the entry of phytotoxic compounds

535 such as Cd into plant roots (Yu et al., 2020), and reduce their uptake by roots (Bao et

536 al., 2019). The major constituents of iron plaques are reported to be fibrous iron ore

(γ-FeOOH) and needle iron ore (α-FeOOH), which easily incorporate Cd via hydroxyl groups (Chen et al., 2017). Significant increase in iron plaques content when exposed to Cd alone. The reason may be that the plants were stimulated to promote iron plaque production under a high concentration of Cd stress, thereby alleviating the oxidative stress effect. Similarly, (Wu et al., 2021) demonstrated a positive correlation between the As content and the iron plaques. For the system with Fe-based NPs addition, the total amount of iron plaques was further increased with increasing dose of Fe-based NPs, indicating that the rise in iron plaques might be a fundamental cause of the decline in Cd content in rice. It is noteworthy that the effect of reddish-brown iron plaques caused by 200 mg/L nFe_2O_3 was seen to be more pronounced in the pictures (**Figure 1G**), yet the highest iron concentration in the DCB extracts was in the nZVI group. Some of the NPs are difficult to be washed by running water or have entered the epidermal plant cells. Therefore, we speculate that the DCB may extracted this part of iron-based NPs, leading to the high iron plaques concentration. In conclusion, our study suggests that iron plaques are a natural and effective barrier to Cd accumulation in the rice root system and nZVI can further strengthen this barrier to lower Cd accumulation in rice shoots.

4.3 Regulation of iron transport protein expression by NPs

The mechanism was further determined by measuring the genes for Cd uptake and translocation. We found that the expression of three genes, OsNRAMP5, OsHMA3, and OsIRT1, returned to control levels after nHA and nZVI treatments, indicating that Cd uptake was effectively inhibited. However, these three genes were further elevated after exposure to nFe_2O_3, which is consistent with the results of Cd accumulation in rice. The release of Fe ions from nFe_2O_3 is likely a reason for the increase of the expressions of these proteins (**Figure S8A**). Importantly, the protein expression data align with the low Cd accumulation in rice shoots exposed to Cd+NPs, as compared to Cd treatment. The expression of LCT1 in shoots showed a striking increase under nFe2O3 induction, emphasizing the overload of Fe transporter proteins (LCT1). It is speculated that the release of Fe ions from Fe-based NPs may have caused the up-

regulation of *LCT* in shoots due to the positive correlation of *LCT1* expression and iron ions(Huang et al., 2022). These results demonstrated that nZVI and nHA NPs suppressed Cd-induced expression of Cd uptake and transport genes in rice roots under Cd stress. Therefore, 200 mg/L nZVI could be an efficient agent to lower Cd accumulation in rice seedlings.

5 Conclusions

Exposure to the three NPs alone did not have any significant adverse effects on rice development, except for high concentrations of nHA. Under Cd stress, the amendment of all three NPs significantly reduced the phytotoxicity of Cd in rice. The NPs reduced the bioavailability of Cd and mitigated its phytotoxicity a range of mechanisms including adsorption, promotion of iron plaque formation, activating plant antioxidant defense systems, regulation of PC production, hormone homeostasis, mineral homeostasis, and Cd transporter protein expression. In this study, nZVI at 200 mg/L was the strongest in decreasing phytotoxicity and Cd accumulation in rice, mainly by enhancing Cd chelation in vesicles and reducing Cd transport capacity.. Thus, nZVI serves as a sustainable and safe nano-strategy to reduce the accumulation of Cd in food crops. Since Cd accumulation in seeds is a significant human health concern, the findings in this study offer important implications for the influence of nanotechnology on food safety. The present study elucidated the mechanisms of altered Cd uptake, translocation and accumulation by three different NPs and highlighted their potential roles in lowering Cd risks from food consumption. More work focusing on the interaction of NPs and heavy metals in complex environmental media over long periods will further illustrate the potential of this novel sustainable agricultural strategy.

Author contributions

Pingfan Zhou: Writing - Original Draft, Visualization, Conceptualization. Peng Zhang - Methodology, Review & Editing, Conceptualization. Mengke He, Yu Cao, Muhammad Adeel, Noman Shakoor, Yaqi Jiang, Weichen Zhao, Yuanbo Li, Mingshu Li, Azeem Imran, Like Jia, Xingmao Ma, Iseult Lynch: Writing - Review & Editing. Yukui Rui: Writing - Review & Editing, Supervision.

Conflict of interest

The authors declare no conflict of interest.

References

Adeel, M., Shakoor, N., Hussain, T., Azeem, I., Zhou, P., Zhang, P., Hao, Y., Rinklebe, J., Rui, Y., 2021. Bio-interaction of nano and bulk lanthanum and ytterbium oxides in soil system: Biochemical, genetic, and histopathological effects on Eisenia fetida. J. Hazard. Mater. 415, 125574. 10.1016/j.jhazmat.2021.125574.

Adrees, M., Khan, Z.S., Ali, S., Hafeez, M., Khalid, S., Ur Rehman, M.Z., Hussain, A., Hussain, K., Shahid Chatha, S.A., Rizwan, M., 2020a. Simultaneous mitigation of cadmium and drought stress in wheat by soil application of iron nanoparticles. Chemosphere 238, 124681. 10.1016/j.chemosphere.2019.124681.

Adrees, M., Khan, Z.S., Ali, S., Hafeez, M., Khalid, S., Ur Rehman, M.Z., Hussain, A., Hussain, K., Shahid Chatha, S.A., Rizwan, M., 2020b. Simultaneous mitigation of cadmium and drought stress in wheat by soil application of iron nanoparticles. Chemosphere 238, 124681. https://doi.org/10.1016/j.chemosphere.2019.124681.

Ali, A., Shah, T., Ullah, R., Zhou, P., Guo, M., Ovais, M., Tan, Z., Rui, Y., 2021. Review on Recent Progress in Magnetic Nanoparticles: Synthesis, Characterization, and Diverse Applications. Frontiers in chemistry 9, 629054. 10.3389/fchem.2021.629054.

Altaf, M.A., Shahid, R., Ren, M., Naz, S., Altaf, M.M., Khan, L.U., Tiwari, R.K., Lal, M.K., Shahid, M.A., Kumar, R., 2022. Melatonin improves drought stress tolerance of tomato by modulating plant growth, root architecture, photosynthesis, and antioxidant defense system. Antioxidants 11, 309.

Amaral, D.C., Lopes, G., Guilherme, L.R.G., Seyfferth, A.L., 2017. A New Approach to Sampling Intact Fe Plaque Reveals Si-Induced Changes in Fe Mineral Composition and Shoot As in Rice. Environ. Sci. Technol. 51, 38-45. 10.1021/acs.est.6b03558.

Anjum, M., Miandad, R., Waqas, M., Gehany, F., Barakat, M.A., 2019. Remediation of wastewater using various nano-materials. Arab. J. Chem. 12, 4897-4919. 10.1016/j.arabjc.2016.10.004.

Bao, Y., Pan, C., Liu, W., Li, Y., Ma, C., Xing, B., 2019. Iron plaque reduces cerium uptake and translocation in rice seedlings (Oryza sativa L.) exposed to CeO2 nanoparticles with different sizes. Sci. Total Environ. 661, 767-777. https://doi.org/10.1016/j.scitotenv.2019.01.181.

635   Brookbank, B.P., Patel, J., Gazzarrini, S., Nambara, E., 2021. Role of Basal ABA in Plant Growth and
636   Development. GENES 12. 10.3390/genes12121936.

637   Cao, X., Yue, L., Wang, C., Luo, X., Zhang, C., Zhao, X., Wu, F., White, J.C., Wang, Z., Xing, B.,
638   2022. Foliar Application with Iron Oxide Nanomaterials Stimulate Nitrogen Fixation, Yield, and
639   Nutritional Quality of Soybean. ACS Nano 16, 1170-1181. 10.1021/acsnano.1c08977.

640   Chen, Z., Tang, Y., Yao, A., Cao, J., Wu, Z., Peng, Z., Wang, S., Xiao, S., Baker, A.J.M., Qiu, R.,
641   2017. Mitigation of Cd accumulation in paddy rice (Oryza sativa L.) by Fe fertilization. Environ.
642   Pollut. 231, 549-559. https://doi.org/10.1016/j.envpol.2017.08.055.

643   Cui, Y., Zhang, X., Zhu, Y., 2008. Does copper reduce cadmium uptake by different rice genotypes? J.
644   Environ. Sci.-China 20, 332-338. https://doi.org/10.1016/S1001-0742(08)60052-2.

645   Gao, Y., Wahi, R., Kan, A.T., Falkner, J.C., Colvin, V.L., Tomson, M.B., 2004. Adsorption of
646   cadmium on anatase nanoparticles effect of crystal size and pH. Langmuir 20, 9585-9593.

647   Gong, X., Huang, D., Liu, Y., Zeng, G., Wang, R., Wan, J., Zhang, C., Cheng, M., Qin, X., Xue, W.,
648   2017. Stabilized Nanoscale Zerovalent Iron Mediated Cadmium Accumulation and Oxidative
649   Damage of Boehmeria nivea (L.) Gaudich Cultivated in Cadmium Contaminated Sediments.
650   Environ. Sci. Technol. 51, 11308-11316. 10.1021/acs.est.7b03164.

651   Guha, T., Barman, S., Mukherjee, A., Kundu, R., 2020. Nano-scale zero valent iron modulates Fe/Cd
652   transporters and immobilizes soil Cd for production of Cd free rice. Chemosphere 260, 127533.
653   https://doi.org/10.1016/j.chemosphere.2020.127533.

654   Huang, G., Pan, D., Wang, M., Zhong, S., Huang, Y., Li, F., Li, X., Xing, B., 2022. Regulation of iron
655   and cadmium uptake in rice roots by iron(iii) oxide nanoparticles: insights from iron plaque
656   formation, gene expression, and nanoparticle accumulation. Environmental Science: Nano 9, 4093-
657   4103. 10.1039/D2EN00487A.

658   Hussain, A., Ali, S., Rizwan, M., Rehman, M.Z.U., Qayyum, M.F., Wang, H., Rinklebe, J., 2019.
659   Responses of wheat (Triticum aestivum) plants grown in a Cd contaminated soil to the application
660   of   iron   oxide   nanoparticles.   Ecotox.   Environ.   Safe.   173,   156-164.
661   https://doi.org/10.1016/j.ecoenv.2019.01.118.

662   Hussain, A., Ali, S., Rizwan, M., Ur Rehman, M.Z., Qayyum, M.F., Wang, H., Rinklebe, J., 2019.
663   Responses of wheat (Triticum aestivum) plants grown in a Cd contaminated soil to the application
664   of iron oxide nanoparticles. Ecotox. Environ. Safe. 173, 156-164.

665   International Agency for Research on Cancer, 2012. Agents classified by the IARC Monographs.
666   https://monographs.iarc.who.int/agents-classified-by-the-iarc/ (accessed2021).

667   Ji, Y., Zhou, Y., Ma, C., Feng, Y., Hao, Y., Rui, Y., Wu, W., Gui, X., Le, V.N., Han, Y., Wang, Y.,
668   Xing, B., Liu, L., Cao, W., 2017. Jointed toxicity of TiO 2 NPs and Cd to rice seedlings: NPs
669   alleviated Cd toxicity and Cd promoted NPs uptake. Plant Physiol. Bioch. 110, 82.

670   Kandhol, N., Singh, V.P., White, J.C., Phan Tran, L., Tripathi, D.K., 2022. Plant growth hormones and
671   nanomaterial interface: Linking development to defence. Plant Cell Physiol.

672   Kaya, C., _enbayram, M., Akram, N.A., Ashraf, M., Alyemeni, M.N., Ahmad, P., 2020. Sulfur-
673   enriched leonardite and humic acid soil amendments enhance tolerance to drought and phosphorus
674   deficiency stress in maize (Zea mays L.). Sci. Rep.-UK 10, 6432. 10.1038/s41598-020-62669-6.

675   Kaya, C., Ashraf, M., Alyemeni, M.N., Ahmad, P., 2020. Responses of nitric oxide and hydrogen
676   sulfide in regulating oxidative defence system in wheat plants grown under cadmium stress.
677   Physiol. Plantarum 168, 345-360. https://doi.org/10.1111/ppl.13012.

678   Kaya, C., Okant, M., Ugurlar, F., Alyemeni, M.N., Ashraf, M., Ahmad, P., 2019. Melatonin-mediated

nitric oxide improves tolerance to cadmium toxicity by reducing oxidative stress in wheat plants. Chemosphere 225, 627-638. https://doi.org/10.1016/j.chemosphere.2019.03.026.

Kaya, C., Polat, T., Ashraf, M., Kaushik, P., Alyemeni, M.N., Ahmad, P., 2021. Endogenous nitric oxide and its potential sources regulate glutathione-induced cadmium stress tolerance in maize plants. Plant Physiol. Bioch. 167, 723-737. https://doi.org/10.1016/j.plaphy.2021.08.030.

Kaya, C., Sarıoglu, A., Ashraf, M., Alyemeni, M.N., Ahmad, P., 2022. The combined supplementation of melatonin and salicylic acid effectively detoxifies arsenic toxicity by modulating phytochelatins and nitrogen metabolism in pepper plants. Environ. Pollut. 297, 118727. https://doi.org/10.1016/j.envpol.2021.118727.

Lee, S., An, G., 2009. Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice. Plant, Cell & Environment 32, 408-416. https://doi.org/10.1111/j.1365-3040.2009.01935.x.

Li, M., Zhang, P., Adeel, M., Guo, Z., Chetwynd, A.J., Ma, C., Bai, T., Hao, Y., Rui, Y., 2021. Physiological impacts of zero valent iron, Fe3O4 and Fe2O3 nanoparticles in rice plants and their potential as Fe fertilizers. Environ. Pollut. 269, 116134. https://doi.org/10.1016/j.envpol.2020.116134.

Liu, C., Wang, L., Yin, J., Qi, L., Feng, Y., 2018. Combined Amendments of Nano-hydroxyapatite Immobilized Cadmium in Contaminated Soil-Potato (Solanum tuberosum L.) System. B. Environ. Contam. Tox. 100, 581-587. 10.1007/s00128-018-2299-3.

Liu, W.J., Zhu, Y.G., Hu, Y., Williams, P.N., Gault, A.G., Meharg, A.A., Charnock, J.M., Smith, F.A., 2006. Arsenic Sequestration in Iron Plaque, Its Accumulation and Speciation in Mature Rice Plants (Oryza Sativa L.). Environ. Sci. Technol. 40, 5730-5736. 10.1021/es060800v.

Lu, C., Zhang, L., Tang, Z., Huang, X., Ma, J.F., Zhao, F., 2019. Producing cadmium-free Indica rice by overexpressing OsHMA3. Environ. Int. 126, 619-626. https://doi.org/10.1016/j.envint.2019.03.004.

Lu, H., Li, Z., Wu, J., Shen, Y., Li, Y., Zou, B., Tang, Y., Zhuang, P., 2017. Influences of calcium silicate on chemical forms and subcellular distribution of cadmium in Amaranthus hypochondriacus L. Sci. Rep.-UK 7, 40583. 10.1038/srep40583.

Lu, Y., Song, S., Wang, R., Liu, Z., Meng, J., Sweetman, A.J., Jenkins, A., Ferrier, R.C., Li, H., Luo, W., Wang, T., 2015. Impacts of soil and water pollution on food safety and health risks in China. Environ. Int. 77, 5-15. 10.1016/j.envint.2014.12.010.

Ma, C., Borgatta, J., Hudson, B.G., Tamijani, A.A., De La Torre-Roche, R., Zuverza-Mena, N., Shen, Y., Elmer, W., Xing, B., Mason, S.E., Hamers, R.J., White, J.C., 2020. Advanced material modulation of nutritional and phytohormone status alleviates damage from soybean sudden death syndrome. Nat. Nanotechnol. 15, 1033-1042. 10.1038/s41565-020-00776-1.

Ma, C., Hao, Y., Zhao, J., Zuverza-Mena, N., Meselhy, A.G., Dhankher, O.P., Rui, Y., White, J.C., Xing, B., 2021. Graphitic Carbon Nitride (C3N4) Reduces Cadmium and Arsenic Phytotoxicity and Accumulation in Rice (Oryza sativa L.). Nanomaterials 11, 839. 10.3390/nano11040839.

Ma, X., Sharifan, H., Dou, F., Sun, W., 2020. Simultaneous reduction of arsenic (As) and cadmium (Cd) accumulation in rice by zinc oxide nanoparticles. Chem. Eng. J. 384, 123802. https://doi.org/10.1016/j.cej.2019.123802.

Mano, Y., Nemoto, K., 2012. The pathway of auxin biosynthesis in plants. J. Exp. Bot. 63, 2853-2872. 10.1093/jxb/ers091.

Mansoor, S., Khan, T., Farooq, I., Shah, L.R., Sharma, V., Sonne, C., Rinklebe, J., Ahmad, P., 2022. Drought and global hunger: biotechnological interventions in sustainability and management.

723   Planta 256, 97. 10.1007/s00425-022-04006-x.

724 Michálková, Z., Martínez-Fernández, D., Komárek, M., 2017. Interactions of two novel stabilizing
725   amendments with sunflower plants grown in a contaminated soil. Chemosphere 186, 374-380.

726 Pishkar, L., Yousefi, S., Iranbakhsh, A., 2022. Foliar application of Zinc oxide nanoparticles alleviates
727   cadmium toxicity in purslane by maintaining nutrients homeostasis and improving the activity of
728   antioxidant enzymes and glyoxalase system. Ecotoxicology 31, 667-678. 10.1007/s10646-022-
729   02533-7.

730 Poole Jr, C.P., Owens, F.J., 2003. Introduction to nanotechnology. John Wiley & Sons.

731 Qin, S., Liu, H., Nie, Z., Rengel, Z., Gao, W., Li, C., Zhao, P., 2020. Toxicity of cadmium and its
732   competition with mineral nutrients for uptake by plants: A review. Pedosphere 30, 168-180.
733   https://doi.org/10.1016/S1002-0160(20)60002-9.

734 Rehman, M.Z.U., Rizwan, M., Hussain, A., Saqib, M., Ali, S., Sohail, M.I., Shafiq, M., Hafeez, F.,
735   2018. Alleviation of cadmium (Cd) toxicity and minimizing its uptake in wheat (Triticum aestivum)
736   by using organic carbon sources in Cd-spiked soil. Environ. Pollut. 241, 557-565.
737   10.1016/j.envpol.2018.06.005.

738 Rizwan, M., Ali, S., Adrees, M., Rizvi, H., Zia-Ur-Rehman, M., Hannan, F., Qayyum, M.F., Hafeez, F.,
739   Ok, Y.S., 2016. Cadmium stress in rice: toxic effects, tolerance mechanisms, and management: a
740   critical review. Environ. Sci. Pollut. R. 23, 17859-17879. 10.1007/s11356-016-6436-4.

741 Rossi, L., Sharifan, H., Zhang, W., Schwab, A.P., Ma, X., 2018. Mutual effects and in planta
742   accumulation of co-existing cerium oxide nanoparticles and cadmium in hydroponically grown
743   soybean (Glycine max (L.) Merr.). Environmental Science: Nano 5, 150-157.
744   10.1039/C7EN00931C.

745 Rossi, L., Zhang, W., Schwab, A.P., Ma, X., 2017. Uptake, Accumulation, and in Planta Distribution of
746   Coexisting Cerium Oxide Nanoparticles and Cadmium in Glycine max (L.) Merr. . Environ. Sci.
747   Technol. 51, 12815-12824. 10.1021/acs.est.7b03363.

748 Ryan, P.R., Delhaize, E., Jones, D.L., 2001. Function and mechanism of organic anion exudation from
749   plant roots. Annu. Rev. Plant Biol. 52, 527.

750 Sebastian, A., Prasad, M.N.V., 2016. Iron plaque decreases cadmium accumulation in Oryza sativa L.
751   and serves as a source of iron. Plant Biology 18, 1008-1015. https://doi.org/10.1111/plb.12484.

752 Shakoor, N., Adeel, M., Ahmad, M.A., Hussain, M., Azeem, I., Zain, M., Zhou, P., Li, Y., Xu, M., Rui,
753   Y., 2023. Environment relevant concentrations of lithium influence soybean development via
754   metabolic reprogramming. J. Hazard. Mater. 441, 129898.
755   https://doi.org/10.1016/j.jhazmat.2022.129898.

756 Shakoor, N., Adeel, M., Zain, M., Zhang, P., Ahmad, M.A., Farooq, T., Zhou, P., Azeem, I., Rizwan,
757   M., Guo, K., Jilani, G., Ahmar, S., Maqbool, S., Ming, X., Rui, Y., 2022. Exposure of cherry
758   radish (Raphanus sativus L. var. Radculus Pers) to iron-based nanoparticles enhances its
759   nutritional quality by trigging the essential elements. NanoImpact 25, 100388.
760   https://doi.org/10.1016/j.impact.2022.100388.

761 Tagawa, K., Tsujimoto, H.Y., Arnon, D.I., 1963. Role of chloroplast ferredoxin in the energy
762   conversion process of photosynthesis. Proceedings of the National Academy of Sciences 49, 567-
763   572.

764 Tang, L., Mao, B., Li, Y., Lv, Q., Zhang, L., Chen, C., He, H., Wang, W., Zeng, X., Shao, Y., Pan, Y.,
765   Hu, Y., Peng, Y., Fu, X., Li, H., Xia, S., Zhao, B., 2017. Knockout of OsNramp5 using the
766   CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. Sci.

767   Rep.-UK 7, 14438. 10.1038/s41598-017-14832-9.

768   The Ministry Of Environmental Protection, The Ministry Of Land Resources, 2014. Bulletin of
769   national   soil   pollution   survey.
770   https://www.mee.gov.cn/gkml/sthjbgw/qt/201404/t20140417_270670.html (accessed.

771   Tripathi, D., Singh, M., Pandey-Rai, S., 2022. Crosstalk of nanoparticles and phytohormones regulate
772   plant growth and metabolism under abiotic and biotic stress. Plant Stress, 100107.

773   Uraguchi, S., Kamiya, T., Sakamoto, T., Kasai, K., Sato, Y., Nagamura, Y., Yoshida, A., Kyozuka, J.,
774   Ishikawa, S., Fujiwara, T., 2011. Low-affinity cation transporter (OsLCT1) regulates cadmium
775   transport into rice grains. Proceedings of the National Academy of Sciences 108, 20959-20964.
776   10.1073/pnas.1116531109.

777   Van Gestel, C.A., Koolhaas, J.E., 2004. Water‐extractability, free ion activity, and pH explain
778   cadmium sorption and toxicity to Folsomia candida (Collembola) in seven soil‐pH combinations.
779   Environmental Toxicology and Chemistry: An International Journal 23, 1822-1833.

780   Veneklaas, E.J., Lambers, H., Bragg, J., Finnegan, P.M., Lovelock, C.E., Plaxton, W.C., Price, C.A.,
781   Scheible, W.R., Shane, M.W., White, P.J., 2012. Opportunities for improving phosphorus‐use
782   efficiency in crop plants. New phytologist 195, 306-320.

783   Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R.K., Kumar, V., Verma, R.,
784   Upadhyay, R.G., Pandey, M., Sharma, S., 2017. Abscisic Acid Signaling and Abiotic Stress
785   Tolerance in Plants: A Review on Current Knowledge and Future Prospects. Front. Plant Sci. 8,
786   161.

787   Wan, Y., Wang, K., Liu, Z., Yu, Y., Wang, Q., Li, H., 2019. Effect of selenium on the subcellular
788   distribution of cadmium and oxidative stress induced by cadmium in rice (Oryza sativa L.).
789   Environ. Sci. Pollut. R. 26, 16220-16228. 10.1007/s11356-019-04975-9.

790   Wang, J., Yuan, J., Yang, Z., Huang, B., Zhou, Y., Xin, J., Gong, Y., Yu, H., 2009. Variation in
791   Cadmium Accumulation among 30 Cultivars and Cadmium Subcellular Distribution in 2 Selected
792   Cultivars of Water Spinach (Ipomoea aquatica Forsk.). J. Agr. Food Chem. 57, 8942-8949.
793   10.1021/jf900812s.

794   Wang, Y., Wang, L., Ma, C., Wang, K., Hao, Y., Chen, Q., Mo, Y., Rui, Y., 2019. Effects of cerium
795   oxide on rice seedlings as affected by co-exposure of cadmium and salt. Environ. Pollut. 252,
796   1087-1096. https://doi.org/10.1016/j.envpol.2019.06.007.

797   Wang, Y., Zhang, P., Li, M., Guo, Z., Ullah, S., Rui, Y., Lynch, I., 2020. Alleviation of nitrogen stress
798   in rice (Oryza sativa) by ceria nanoparticles. Environmental Science: Nano 7, 2930-2940.
799   10.1039/D0EN00757A.

800   Wang, Z., Tang, J., Zhu, L., Feng, Y., Yue, L., Wang, C., Xiao, Z., Chen, F., 2022. Nanomaterial-
801   induced modulation of hormonal pathways enhances plant cell growth. Environmental Science:
802   Nano 9, 1578-1590. 10.1039/D2EN00251E.

803   Wani, S.H., Kumar, V., Shriram, V., Sah, S.K., 2016. Phytohormones and their metabolic engineering
804   for   abiotic   stress   tolerance   in   crop   plants.   The   Crop   Journal   4,   162-176.
805   https://doi.org/10.1016/j.cj.2016.01.010.

806   Wu, X., Hu, J., Wu, F., Zhang, X., Wang, B., Yang, Y., Shen, G., Liu, J., Tao, S., Wang, X., 2021.
807   Application of TiO2 nanoparticles to reduce bioaccumulation of arsenic in rice seedlings (Oryza
808   sativa   L.):   A   mechanistic   study.   J.   Hazard.   Mater.   405,   124047.
809   https://doi.org/10.1016/j.jhazmat.2020.124047.

810   Xiong, L., Wang, P., Hunter, M.N., Kopittke, P.M., 2018. Bioavailability and movement of

hydroxyapatite nanoparticles (HA-NPs) applied as a phosphorus fertiliser in soils. Environmental Science: Nano 5, 2888-2898. 10.1039/C8EN00751A.

Yadav, S.K., 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S. Afr. J. Bot. 76, 167-179. https://doi.org/10.1016/j.sajb.2009.10.007.

Yang, M., Zhang, Y., Zhang, L., Hu, J., Zhang, X., Lu, K., Dong, H., Wang, D., Zhao, F., Huang, C., Lian, X., 2014. OsNRAMP5 contributes to manganese translocation and distribution in rice shoots. J. Exp. Bot. 65, 4849-4861. 10.1093/jxb/eru259.

Yu, H., Wu, Y., Huang, H., Zhan, J., Wang, K., Li, T., 2020. The predominant role of pectin in binding Cd in the root cell wall of a high Cd accumulating rice line (Oryza sativa L.). Ecotox. Environ. Safe. 206, 111210. https://doi.org/10.1016/j.ecoenv.2020.111210.

Zhang, P., Guo, Z., Luo, W., Monikh, F.A., Xie, C., Valsami-Jones, E., Lynch, I., Zhang, Z., 2020. Graphene Oxide-Induced pH Alteration, Iron Overload, and Subsequent Oxidative Damage in Rice (Oryza sativa L.): A New Mechanism of Nanomaterial Phytotoxicity. Environ. Sci. Technol. 54, 3181-3190. 10.1021/acs.est.9b05794.

Zhang, P., Guo, Z., Monikh, F.A., Lynch, I., Valsami-Jones, E., Zhang, Z., 2021. Growing Rice (Oryza sativa) Aerobically Reduces Phytotoxicity, Uptake, and Transformation of CeO2 Nanoparticles. Environ. Sci. Technol. 55, 8654-8664. 10.1021/acs.est.0c08813.

Zhang, P., Wu, X., Guo, Z., Yang, X., Hu, X., Lynch, I., 2021. Stress Response and Nutrient Homeostasis in Lettuce (Lactuca sativa) Exposed to Graphene Quantum Dots Are Modulated by Particle Surface Functionalization. Advanced Biology 5, 2000778. https://doi.org/10.1002/adbi.202000778.

Zhao, F., Ma, Y., Zhu, Y., Tang, Z., Mcgrath, S.P., 2015. Soil Contamination in China: Current Status and Mitigation Strategies. Environ. Sci. Technol. 49, 750-759. 10.1021/es5047099.

Zhao, L., Bai, T., Wei, H., Gardea-Torresdey, J.L., Keller, A., White, J.C., 2022. Nanobiotechnology-based strategies for enhanced crop stress resilience. Nature Food 3, 829-836. 10.1038/s43016-022-00596-7.

Zhao, W., Adeel, M., Zhang, P., Zhou, P., Huang, L., Zhao, Y., Ahmad, M.A., Shakoor, N., Lou, B., Jiang, Y., Lynch, I., Rui, Y., 2022. A critical review on surface-modified nano-catalyst application for the photocatalytic degradation of volatile organic compounds. Environmental Science: Nano 9, 61-80. 10.1039/D1EN00955A.

Zhou, P., Adeel, M., Guo, M., Ge, L., Shakoor, N., Li, M., Li, Y., Wang, G., Rui, Y., 2022. Characterisation of biochar produced from two types of chestnut shells for use in remediation of cadmium- and lead-contaminated soil. Crop and pasture science. 10.1071/CP21297.

Zhou, P., Adeel, M., Shakoor, N., Guo, M., Hao, Y., Azeem, I., Li, M., Liu, M., Rui, Y., 2021. Application of Nanoparticles Alleviates Heavy Metals Stress and Promotes Plant Growth: An Overview. Nanomaterials 11, 26. 10.3390/nano11010026.

Zhou, P., Long, B., Wang, R., Jiang, Y., Zhao, W., Li, Y., Li, M., Guo, Z., Zhang, P., Rui, Y., 2022. Increase in the active ingredients of traditional Chinese medicine Isatis indigotica through iron nanoparticles supplementation versus carbon nanotubes: a comparative study. ENVIRONMENTAL SCIENCE-NANO.