UNIVERSITY^{OF} BIRMINGHAM University of Birmingham Research at Birmingham

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

Zhou, Pingfan; Zhang, Peng; He, Mengke; Cao, Yu; Adeel, Muhammad; Shakoor, Noman; Jiang, Yaqi; Zhao, Weichen; Li, Yuanbo; Li, Mingshu; Azeem, Imran; Jia, Like; Rui, Yukui; Ma, Xingmao; Lynch, Iseult

DOI: 10.1016/j.envpol.2023.121063

License: Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version Peer reviewed version

Citation for published version (Harvard):

Zhou, P, Zhang, P, He, M, Cao, Y, Adeel, M, Shakoor, N, Jiang, Y, Zhao, W, Li, Y, Li, M, Azeem, I, Jia, L, Rui, Y, Ma, X & Lynch, I 2023, 'Iron-based nanomaterials reduce cadmium toxicity in rice (*Oryza sativa L.*) by modulating phytohormones, phytochelatin, cadmium transport genes and iron plaque formation', *Environmental Pollution*, vol. 320, 121063. https://doi.org/10.1016/j.envpol.2023.121063

Link to publication on Research at Birmingham portal

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

Users may freely distribute the URL that is used to identify this publication.
Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private

•Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

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

- 5 Pingfan Zhou^{*a*, 1}, Peng Zhang^{*b*, *c*, 1}, Mengke He^{*a*}, Yu Cao^{*a*}, Muhammad Adeel^{*d*},
- 6 Noman Shakoor^a, Yaqi Jiang^a, Weichen Zhao^a, Yuanbo Li^a, Mingshu Li^a, Imran
- 7 Azeem^a, Like Jia^a, Yukui Rui^{a,*}, Xingmao Ma^e, Iseult Lynch^c
- ^a Beijing Key Laboratory of Farmland Soil Pollution Prevention and Remediation,
- 9 College of Resources and Environmental Sciences, China Agricultural University,
- 10 Beijing 100193, China
- ^b Department of Environmental Science and Engineering, University of Science and
- 12 Technology of China, Hefei 230026, China
- ^c School of Geography, Earth and Environmental Sciences, University of Birmingham,
- 14 Edgbaston, Birmingham, B15 2TT, UK
- ^d BNU-HKUST Laboratory of Green Innovation, Advanced Institute of Natural
- 16 Sciences, Beijing Normal University at Zhuhai 519087, China
- ^e Zachry Department of Civil and Environmental Engineering, Texas A&M University,
- 18 TAMU 3136, College Station, TX 77843-3136, USA
- ¹ The two authors contributed equally to the manuscript.
- 20 * Corresponding author information:
- 21 Email: ruiyukui@163.com

22 Abstract

Rice is known to accumulate cadmium (Cd) in its grains, causing a severe threat to billions of 23 people worldwide. The possible phytotoxicity and mechanism of 50-200 mg/L hydroxyapatite NPs 24 (nHA), iron oxide NPs (nFe₂O₃) or nano zero valent iron (nZVI) co-exposed with Cd (100 μ M) in 25 rice seedlings were investigated. Three types of nanoparticles significantly reduced the 26 bioaccumulation of Cd in rice shoots by 16-63%, with nZVI showing the greatest effect, followed by 27 nHA and nFe₂O₃. A decrease in Cd content in the roots was observed only in the nZVI treatment, 28 with values ranging from 8-19%. Correspondingly, nZVI showed the best results in promoting plant 29 growth, increasing rice plant height, shoot and root biomass by 13%, 29% and 42%. In vitro studies 30 showed that nZVI reduced the content of Cd in the solution by 20-52% through adsorption, which 31 might have contributed to the immobilization of Cd in root. Importantly, the nZVI treatment resulted 32 in 267% more iron plaques on the root surface, which acted as a barrier to hinder the entry of Cd. 33 Moreover, all three nanoparticles significantly reduced the oxidative stress induced by Cd by 34 regulating phytohormones, phytochelatin, inorganic homeostasis and the expression of genes 35 associated with Cd uptake and transport. Overall, this study elucidates for the first time the multiple 36 37 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. 38

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

Abbreviations								
Abbreviation	Full name							
NPs	Nanoparticles							
TEM	Transmission Electron Microscope							
CAS	Chinese Academy of Sciences Deionized water							
DI								
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							

41 **1 Introduction**

Human health risks caused by heavy metals have attracted attention worldwide (Lu et al., 2015). 42 Cadmium (Cd) is a toxic heavy metal commonly found in agricultural soils (Rehman et al., 2018). 43 The World Health Organization (WHO) designated Cd and Cd-containing compounds as group I 44 carcinogens due to their high toxicity and bioaccumulation potential (International Agency for 45 46 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 47 agricultural soils is 7.0%, which is the highest inorganic pollutant (The Ministry Of Environmental 48 Protection and The Ministry Of Land Resources, 2014; Zhao et al., 2015). Cd in soil displays a long 49 decomposition cycle, high mobility and toxicity, resulting in reduced seed germination, plant growth 50 inhibition and yield reduction (Kaya et al., 2019; Kaya et al., 2020; Rossi et al., 2018). Moreover, 51 phytoaccumulation of Cd in the food chain may endanger human health (Zhou et al., 2022). 52 Therefore, there is an urgent need of innovative and sustainable strategies to control the absorption of 53 cadmium in edible crops. 54

Nanotechnology is the convergence of various sciences to provide disruptive technologies for 55 56 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 57 their unique chemical and physical properties (Ali et al., 2021; Mansoor et al., 2022; Zhao et al., 58 2022). Compared with traditional materials, NPs exhibit greater adsorption capacity, and catalytic 59 and magnetic properties (Adeel et al., 2021; Shakoor et al., 2022). A recent report has revealed that 60 the interaction between Cd and NPs and may alter the absorption, transfer and accumulation of Cd in 61 rice (Zhou et al., 2021). Ji et al., (2017) reported that 100 mg/L of titanium dioxide alleviated the 62 phytotoxicity of Cd, mainly because Cd was adsorbed by TiO₂. Similarly, (Wang et al., 2019) 63 demonstrated that CeO₂ NPs alleviated the Cd stress and mitigated DNA damage in rice. 64

65 Cd is non-essential and no specific transporter proteins were identified for Cd uptake by rice 66 (Tang et al., 2017). Instead, Cd uptake and transfer is mainly through the iron transporter, which 67 involves *NRAMP* and *IRT* family transporters (Yang et al., 2014). Therefore, the competition 68 between Cd and Fe absorb may cause an imbalance of Fe homeostasis in rice, leading to chlorosis of 69 leaves (Zhang et al., 2020). Improving Fe homeostasis in plants using iron-based NPs thus 70 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 78 (nFe₂O₃)) and one natural inorganic mineral NPs (hydroxyapatite, nHA, Ca₁₀(PO₄)₆(OH)₂) were 79 chosen to evaluate their impact on Cd accumulation in rice tissues due to their known beneficial 80 effects on plants. For example, a whole life cycle culture study showed that Fe₂O₃ NPs reduced the 81 harm of Cd to wheat growth and yield (Adrees et al., 2020a). Due to its high elemental P, nHA 82 resulted in a 16.5 and 8-fold increase in aboveground and root fresh biomass of sunflower grown in 83 phosphorus-deficient soils (Xiong et al., 2018). In addition, soil application of nHA promoted potato 84 growth and reduced the Cd content in tubers by 17% compared to Cd treatment alone (Liu et al., 85 2018). All three NPs exhibited excellent biocompatibility at environment-relevant concentrations and 86 were inexpensive, making them promising tools for Cd-contaminated soil. However, comprehensive 87 studies concerning the physiological and biochemical effects of NPs and Cd on rice are still lacking. 88

In this study, we hypothesized that the addition of NPs could alter the uptake, translocation, and 89 subsequent effects of Cd on rice, the most important food crop in many Asian countries (Zhang et al., 90 2021). Specifically, we investigated the effects of different NPs on rice growth under Cd stress by 91 measuring a range of phenotypic and physiological parameters, including the biomass, root 92 morphology, photosynthetic system, and inorganic nutrients. The stress status and plant tolerance to 93 stress were also assessed by measuring the response of antioxidant systems and phytohormones in 94 plants. In addition, the adsorption capacity of different NPs for Cd, the blockage of Cd by iron 95 plaques and the expression of Cd transport genes were determined and their correlations with the 96 observed biological effects were investigated. 97

98 2. Materials and methods

99 2.1 Characterization of NPs

Fe₂O₃ 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**.

105

106 *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.

111

112 2.3 Plant culture and NPs treatment

113 Seeds (Y Liangyou 900) of rice (Oryza sativa L.) were acquired from the CAS. Rice seeds were disinfected with 10% H₂O₂ for 20 min and flushed completely using 114 DI water. The seeds were placed evenly on a plastic tray containing a layer of gauze, 115 then covered with a sheet of filter paper and immersed in 15 ml of DI water. The tray 116 was then sealed with a film tray to avoid water loss. And germinate in a climatic 117 incubator under darkness for 7 days (25°C). Similarly sized seedlings were anchored 118 in sponge and planted into a plastic bottle with 250 ml ¹/₄ strength Kimura B. 119 Seedlings were cultivated in a climatic incubator (25/22 °C and a 16 h photoperiod). 120 121 After 7 days of acclimation, rice seedlings were individually exposed to 50 and 200 mg/L nHA, nFe₂O₃ or nZVI for 14 days or 100 µM Cd + NPs. Seedlings treated with 122 100 µM Cd without addition of NPs were used as a control. Seedlings without any 123 treatment were used as blank control. The Kimura B was added regularly to an initial 124 volume of 250 ml every other day, followed by the determination of Cd levels in the 125 hydroponic system. At day 14, all seedlings were harvested and rinsed thoroughly 126 with 10% of nitric acid and DI water to remove the NPs and Cd attached on root 127

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

130

131 *2.4 Root morphology and iron plaque extraction*

An automated scanner (Epson expression 1680, Japan) was used to scan the rice 132 roots, followed by analysis of root parameters using RHIZO software (Pro 2007d, 133 Quebec, Canada). The extraction of iron plaque on the root surface was performed by 134 the citric acid bicarbonate dithionate (DCB) method as described by Liu et al., (Liu et 135 al., 2006). Briefly, fresh roots were soaked in 20 ml of 125 mM NaHCO3 and 30 mM 136 Na₃C₆H₅O₇ mixture, the added 0.4 g Na₂S₂O₄ powder. The mixture containing roots is 137 stirred and placed at 25 °C for 1 hour. The roots were rinsed with DI water, and all 138 eluents were collected and diluted to 50 ml with DI water, followed by Fe and Cd 139 measurements. The digestion and determination methods of elements (Cd and other 140 mineral elements) are shown in Section 1, S2. 141

142

143 2.5 Determination of antioxidative system, metal chelators, chlorophyll content and 144 phytohormones

The phytochelatin (PCs), glutathione (GSH), non-protein thiol (NPTH) that can bind Cd²⁺ 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**.

151

152 2.6 Extraction of subcellular Cd fractions

153 Cells were isolated in three fractions three fractions using centrifugation 154 according to the method of Wan et al., (Wan et al., 2019). Briefly, the tissue samples 155 were homogenized in buffer and centrifuged at different speeds to collect three cell 156 fractions. These three parts were digested with nitric acid and determined by ICP-MS. 157 Details are presented in **Section1**, **S6**. 158

159 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**.

165

166 *2.8 Data analysis*

167 SPSS 20.0 was used to conduct the data analysis. Statistical significance was 168 executed through one-way ANOVA. The average values of each treatment were 169 compared using the Tukey's HSD at p < 0.05 confidence level.

170 **3 Results**

171 3.1 Different impacts of nanoparticles on rice phenotypes under cadmium exposure

172 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 173 fresh weight by 7% (Figure 1D). nZVI showed stronger effects than other NPs both at 174 low and high concentrations, and 200 mg/L of nZVI increased the plant height, shoot 175 weight and root weight by 13%, 29% and 42%, respectively. Cd (100 µM) 176 significantly inhibited the rice seedling growth. Cd treatment reduced the shoot and 177 root lengths, shoot and root biomass by 45%, 23%, 67% and 30%, respectively 178 (Figure 1). The presence of NPs mitigated the phytotoxicity of Cd and improved rice 179 growth parameters (e.g., biomass and root morphology) in a dose-dependent manner 180 (Figure 1E, F and S3). Specifically, all three NPs increased the fresh shoot and root 181 biomass by 58.27%-106.51% and 13.32%-66.52%, respectively, compared to the Cd 182 treatment (Figure 1C, D). Again, nZVI at 200 mg/L showed the strongest positive 183 effects on alleviating the Cd toxicity, resulting in 35%, 17%, 107% and 67% increase 184 in plant height, root length, shoot biomass and root biomass, correspondingly. 185 Furthermore, nHA and nZVI at 200 mg/L also improved all the root parameters 186

(Figure S4). In particular, nZVI at 200mg/L enhanced the total root length, average
root diameter, total surface area and total root volume by 135, 82, 127 and 163%,
respectively.



190

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 \pm 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.

198 *3.2 Nanoparticles regulate photosynthesis, antioxidative system and metal chelators*

Compared with Cd treatment, the addition of NPs enhanced the leaf moisture 199 (77%-127%), Cha (52%-131%), Chb (109%-295%) and SPAD values (39%-82%). 200 High concentration of nZVI (200mg/L) showed the strongest positive effects, 201 resulting in an increase of 127%, 131%, 295% and 82% in leaf moisture, Cha, Chb 202 and SPAD values, respectively (Figure S5). MDA contents in shoots with Cd 203 exposure alone was significantly increased 48% relative to the control, which means 204 205 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 206 25%, respectively. Notably, the addition of NPs further elevated POD and SOD 207 activities by 169%-308% and 91%-209%, and reduced the MDA contents (65%-76%). 208 209 Additional discussions about photosynthesis and enzymatic antioxidant system are provided in the Section1, S9 and S10. These results suggest that NPs treatments 210 enhanced the enzymatic antioxidant systems. Under Cd stress, the non-enzymatic 211 antioxidant system such as GSH and non-protein thiols (NPTH) were not significantly 212 altered by NPs supplement (Figure 2A and 2B), except for ZVI alone at 200 mg/L 213 enhanced NPTH level (98%). Previous evidence has suggested that phytochelatins 214 (PCs) play important roles in the detoxification of Cd in plants (Guha et al., 2020). 215 Interestingly, PCs in rice increased by 208-346% as compared with the control after 216 217 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. 218 Specifically, nZVI at 200 mg/L elevated the content of NPTH and PCs by 88% and 49% 219 compared to the Cd treatment (Figure 2B and 2C). nFe₂O₃ also significantly elevated 220 PC content (73%-84%), but it did not significantly elevate NTPH (24%-46%). The 221 above results indicate that the addition of NPs can modulates the antioxidant system 222 and PCs levels in plants, which protects plant from Cd stress. 223



224

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 \pm SD of 4 replicates. Significant differences at p < 0.05 are indicated by different lowercase letters.

229 3.3 Regulation of phytohormones by nanoparticles

230 Under Cd stress, all phytohormones except for ZR were significantly upregulated compared to the control (Figure 3). The phytohormones displayed similar 231 trends for NPs alone and Cd+NPs except for ABA and ZR. The ABA content 232 significantly decreased by 29-55% when exposed to NPs alone, indicating that the 233 234 exposure to NPs alone did not cause a significant stress response. A previous report demonstrated that excess ABA was associated with plant growth inhibition 235 (Brookbank et al., 2021). However, iron-based NPs resulted in a significant 20-72% 236 increase of ZR, which is considered an antagonist of ABA, and our results are 237 consistent with this trend (Zhang et al., 2021). Overall, a more significant up-238 regulation of ABA and IPA occurred in the case of Cd + NPs. The addition of nZVI 239 and nFe₂O₃ significantly up-regulated ABA by 36%-45% and 25%-44% compared to 240 the Cd control (Figure 3A). Similarly, nZVI and nFe₂O₃ at 200 mg/L resulted in a 241 significant increase in IPA by 52% and 16% (Figure 3B), consistent with the 242 physiological results. In contrast, a decreasing trend was observed for IAA, GA4 and 243 BR after NPs exposure (Figure 3C-3E). Once again, nZVI at 200 mg/L showed the 244 strongest effect, indicating its greatest effectiveness in mitigating Cd stress among the 245 three NPs investigated in this study. For IAA, the addition of 200 mg/L nHA, nZVI 246 and nFe₂O₃ decreased IAA by 17%, 36% and 17%, respectively (Figure 3C). Overall, 247 our results suggest that some NPs can mitigate Cd toxicity, at least in part, by 248



250

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 \pm SD of 4 replicates. Significant differences at p < 0.05 are indicated by different lowercase letters.

255 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

nFe₂O₃ resulted in 131% and 255% increases of Fe in roots (Figure 4A). Similarly, 259 200 mg/L of nHA resulted in 76% and 31% increase in Ca and P in the root. However, 260 the increase of P was not significant (29%-31%), probably due to the high content of 261 P in the nutrient solution. Notably, both nZVI and Fe₂O₃ NPs at 200 mg/L resulted in 262 a 51% increase in Cu content in the roots, with no effect on other inorganic elements 263 except for Fe. Inorganic nutrient homeostasis in shoots was more sensitive to NPs 264 (Figure 4B) than roots. In the treatment of Fe-based NPs, 200 mg/L nZVI resulted in 265 58% and 24% increases in Cu and Zn elements. At 200 mg/L, nHA reduced the 266 content of all five elements in the leaves, i.e., Mg, Ca, Fe, Cu, and Zn by 25%, 37%, 267 65%, 61%, and 28%, respectively. The results suggested that the addition of nHA 268 might have blocked the in-planta transport of inorganic nutrients from underground to 269 aboveground. 270

	A)	Na	Mg	к	Са	Fe	Cu	Zn	Р	_		_
	ск	-0.02	0.51*	0.61*	0.06	-0.33	0.20	0.88*	0.28			
50 mg/kg	nHA	0.24	0.21	-0.16	0.20	0.16	-0.24	-0.18	0.29		-	2
	nZVI	0.31	-0.09	-0.20	-0.16	0.18	0.35*	0.06	0.17			
	nFe ₂ O ₃	0.03	0.15	-0.01	0.06	0.41	-0.09	0.01	0.19		-	1
200 mg/kg	nHA	0.32	-0.14	-0.49*	0.76*	-0.37	-0.14	-0.30	0.31			
	nZVI	0.20	-0.13	-0.22	-0.14	1.31*	0.51*	-0.21	0.21			0
	nFe ₂ O ₃	0.18	-0.09	-0.13	-0.07	2.55 *	0.51*	0.11	-0.03			

	B)	Na	Mg	к	Са	Fe	Cu	Zn	Р		
	ск	0.58	0.33*	0.04	-0.20*	0.17	0.88*	1.00*	0.63*		
50 mg/kg	nHA	0.38	-0.15	-0.05	-0.24*	-0.27	-0.40*	-0.27*	0.11		
	nZVI	0.22	-0.10	0.04	-0.18	-0.41	-0.07	-0.12	0.17		0.5
	nFe ₂ O ₃	0.07	0.07	0.02	-0.03	-0.33	0.01	0.02	0.35		
200 mg/kg	nHA	-0.14	-0.25*	0.001	-0.37 *	-0.65*	-0.51*	-0.28*	0.12		0
	nZVI	0.64	-0.19	-0.09	-0.24*	-0.41	0.58*	0.24*	0.19		
	nFe ₂ O ₃	0.77	0.14	0.03	0.10	-0.26	-0.06	-0.13	0.24		-0.5

271

Figure 4. Thermogram illustrating the homeostasis of inorganic nutrients in rice root (A) and shoot B) modulated by Cd and Cd + NPs after two weeks. Numbers represent the multiplicity of change in content of elements compared to the Cd alone treatment. Negative values denote a decrease in content, while positive values are the opposite. Values indicate the mean \pm SD of 6 replicates. Significant differences compared with the Cd alone control at p < 0.05 (n=4) are indicated by *.

278 3.5 Total cadmium and subcellular distribution in rice tissues

The absorb and subcellular distribution of Cd in plant were further investigated 279 to explore the link between Cd accumulation and plant physiological regulators. Cd 280 accumulation in the roots of plants treated with Cd+NPs was not significantly reduced 281 282 except for the 200 mg/L nZVI treatment (Figure 5A). However, Cd accumulation in the shoots was more significantly reduced after NPs exposure (Figure 5B). 283 Specifically, rice exposed to 200 mg/L nHA and nZVI resulted in a 75% and 80% 284 reduction in Cd in shoots. The effects of different NPs on Cd accumulation of shoots 285 286 upon exposure to Cd are consistent with the results of growth differences in Figure 1. Further investigation of the mechanisms related to the effect of NPs on the absorb and 287 translocation of Cd was conducted by measuring the Cd subcellular distribution. As 288 shown in Figure 5C-D, the majority of Cd was in the soluble fraction (F3, 42–74%), 289 followed by the cell wall fraction (F1, 17–48%) and organelle fraction (F2, 4–13%), 290 both for shoots and roots. The soluble fraction consists mainly of vesicles, which are 291 the main location to preferentially bind Cd to minimize its damage to the cell wall or 292 entry into the organelle (Wang et al., 2009). Compared with Cd alone treatment, nZVI 293 at 200 mg/L significantly enhanced the percentage of Cd in soluble fractions of roots 294 by 20% and decreased the cell wall and organelles fractions by 8% and 12%, 295 respectively (Figure 5C). A similar phenomenon was observed in shoots (Figure 5D), 296 although the difference was not significant. 297



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 \pm SD of 3 replicates. Significant differences at p < 0.05 are indicated by * or different lowercase letters.

304 *3.6 Cadmium sorption in solution and cadmium content in iron plaque*

298

Dynamic chemical changes between Cd and NPs in the nutrient solution may be 305 306 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 307 only 3-8% of the initial Cd content in the nutrient solution was adsorbed on nFe₂O₃ 308 (Figure 6A). However, nHA and nZVI significantly reduced the Cd content in the 309 nutrient solution by 12%-24% and 20%-52%, respectively. The zeta potential of NPs 310 in nutrient solution is critical to the adsorption of NPs. Our data showed that the zero 311 charge points (nHA, nZVI and nFe₂O₃) of the three NPs were 4.4, 4.9 and 7.3, 312

respectively (Figure S2). Therefore, at the beginning of Cd exposure, Cd^{2+} will 313 strongly adsorb to the negatively charged nHA and nZVI through electrostatic 314 interactions. However, nFe₂O₃ has a zeta potential of about +14.5 mV at pH 5.5, and 315 the electrostatic repulsion prevents the approach of Cd and further interactions. 316 Therefore, the adsorption capacity of nFe₂O₃ for Cd is much lower than the other two 317 NPs. In addition to Cd adsorption via NPs, Cd uptake by plants is a primary process 318 leading to the reduction of Cd in nutrient solutions (Figure 6B-D). The uptake of Cd 319 by plants was around 28% of its initial concentration in the Kimura B containing only 320 Cd. With the addition of 200 mg/L nZVI, the plant uptake of Cd only accounted for 6% 321 of its initial content. The remaining 46% and 48% of Cd were either adsorbed by 322 nZVI or retained in the nutrient solution, respectively, emphasizing the essential role 323 of nZVI in reducing the Cd bioavailability (Figure 6C). 324



325

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) nFe₂O₃. 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 \pm SD of 4 replicates. Significant differences at p < 0.05 are indicated by * or different lowercase letters.

332 In order to accommodate the flooded conditions, rice can deliver O_2 from the 333 shoot to the inter-root environment, causing the oxidation of Fe²⁺ and Mn²⁺ to form a

reddish-brown iron oxide film (Iron plaque) (Amaral et al., 2017). The content of iron 334 plaque and the Cd in the plaques were determined to explore the effect of NPs on iron 335 plaque formation and how iron plaques influence the absorption of Cd by roots. The 336 total iron plaque amount was significantly increased by 190% in the Cd exposure 337 alone compared to the blank control. At 200 mg/L nHA, iron plaques were markedly 338 decreased by 58% compared to the Cd control (Figure 6E). Further analysis indicated 339 that the total iron plaque produced on the root surface was positive correlated with the 340 Cd content in the nutrient solution in the Kimura B for the non-Fe-based NPs added 341 system ($R^2 = 0.786$, Figure S9). For the system with Fe-based NPs addition, the total 342 amount of iron plaques was increased with increasing dose of Fe-based NPs. In 343 particular, nZVI significantly increased the mass of iron plaques by 185% and 267% 344 relative to the Cd control, which was in agreement to the results for the Cd content in 345 roots. In the presence of 50 mg/L nHA and nFe₂O₃, Cd levels in iron plaques were 346 significantly reduced by 32-41% relative to the Cd-exposed system alone; the 347 remaining treatments had no significant effect on Cd levels in iron plaques (Figure 348 349 **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 350 fundamental cause of the decline in Cd content in rice. 351

352 3.7 Relative expression of Cd transporters

The relative expression of Cd uptake and translocation-related proteins 353 (OsNRAMP5, OsHMA3, OsIRT1, and OsLCT1) in rice shoots and roots were 354 measured to explore the molecular mechanisms for altered Cd accumulation by NPs 355 (Figure 7). NRAMP5, a resistance- related macrophage protein, is known to be the 356 main transporter protein for Cd^{2+} and Fe^{2+} uptake in rice roots (Yang et al., 2014). 357 Similarly, Cd could be taken up through the Fe (II) transporter, the Fe -regulated 358 transporter 1 (IRT1) in rice roots (Lee and An, 2009). OsHMA3 localized on the 359 tonoplast is a member of the heavy metal ATPase (HMA) family, which could 360 sequester Cd into vacuoles to reduce the transport of Cd to shoots (Lu et al., 2019). 361 Interestingly, we found that the expression of OsNRAMP5, OsHMA3 and OsIRT1 362 genes showed a similar trend both in the roots and shoots. Expression of these three 363 genes in the roots of Cd treated rice was upregulated about 500%, whereas co-364 exposure to nHA and nZVI with Cd restored their expression back to control levels. 365 Surprisingly, co-exposure to nFe₂O₃ with Cd caused an approximately 800% increase 366 in OsNRAMP5, OsHMA3 and OsIRT1 expression in rice roots (Figure 7A, B, C). To 367 better understand the potential effects of NPs on Cd accumulation in the aboveground 368 tissues, the expression of low-affinity cationic transport protein (LCT1) was 369 determined. LCT1 regulates the transport of Cd through the roots to the aboveground 370 tissues (Uraguchi et al., 2011). An approximately 500% increase in expression was 371 observed in roots in all treatments except for the Cd+nFe₂O₃ exposure (Figure 7D). 372 However, nZVI and nFe₂O₃ up-regulated *LCT1* expression in shoots by approximately 373 500% and 1800%, respectively. 374



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 \pm SD of 4 replicates. Significant differences at p < 0.05 are indicated by different lowercase letters.

380 4 Discussion

375

381 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 382 biomass except for nHA, whereas Cd treatments significantly inhibited rice growth. 383 All different types of NPs reduced the phytotoxicity of Cd and improved the growth 384 385 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 386 also observed in one of our previous Fe-based NPs studies, where the "fertilizer effect" 387 and "nano stimulus effect" of nZVI treatment resulted in a rise in chlorophyll, 388 hormone regulation, and enhanced expression of iron transporter protein in rice (Li et 389 al., 2021). To investigate the potential mechanisms of NPs to alleviate Cd stress, we 390 conducted studies on both the physiological and biochemical systems of plants, the 391

formation of iron plaque and the adsorption behavior of NPs.

Cd is known to cause membrane lipid peroxidation and inhibit photosynthesis 393 (Ma et al., 2020; Rossi et al., 2017). Our results suggest that the enhanced biomass 394 and seedling elongation may be partly attributed to the enhanced photosynthesis by 395 NPs treatments. Compared with Cd treatment, the addition of NPs enhanced the leaf 396 moisture, chlorophyll content and antioxidative system. The positive effects of NPs 397 might be related to the release of mineral elements (Fe, P, Ca) which can support 398 399 photosynthesis and plant growth. Compared to conventional ion treatment, the slowrelease effect of nanoparticles can effectively avoid plant toxicity caused by a large 400 supply of ions in a short period of time (Cao et al., 2022). Iron is the central metal for 401 several vital enzymes (e.g., cytochromes) involved in electron transfer during 402 photosynthesis (Tagawa et al., 1963). Similar results were reported previously that 403 foliar application of 20 mg/L nFe₂O₃ increased chlorophyll a, b and carotenoid 404 concentrations by 70%, 139% and 119%, respectively (Hussain et al., 2019). Similarly, 405 406 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 elements indirectly led to an increase in photosynthetic rate, thus enabling rice to 409 accumulate more organic matter (Kaya et al., 2020). 410

Under the Cd stress, plants produce excessive harmful reactive oxygen species 411 (ROS), leading to an increase in MDA content, an important biomarker of lipid 412 peroxidation (Zhou et al., 2022). We found that the MDA content was relatively high 413 414 in rice shoots not treated with NPs due to heavy metal toxicity. The addition of all types of NPs significantly increased the POD and SOD activity, followed by the 415 reduction of MDA contents, indicating that the addition of NPs could further trigger 416 the defense mechanism of antioxidant enzymes. In agreement with our study, nZVI 417 has been shown to increase SOD and POD in sunflower leaves, followed by the 418 decrease of MDA (Michálková et al., 2017). Adress et al., (2020b) also demonstrated 419 that the soil application of iron-based NPs promoted wheat growth and the SOD and 420

POD activities under Cd stress. Recent studies reported the specificity of NPs, not 421 only as key substances for regulating ROS homeostasis, but also for directly 422 423 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 424 after NPs treatment were not significant (NPTH), suggesting that enzymatic 425 antioxidant systems are the main mechanism of action. GSH further decreased after 426 treatment with NPs, demonstrating the involvement of GSH in the elimination of ROS 427 428 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 429 reports (Guha et al., 2020; Kaya et al., 2022). PC could form complexes with toxic 430 metal ions in cytoplasmic solution and subsequently translocate them to the vesicles 431 (Yadav, 2010). A significant rise in PC was observed in the case of Cd + NPs co-432 exposure, with nZVI showing the strongest effect. Notably, the PC-induced rise in Cd 433 content in the vesicles was consistent with that observed in the subcellular fractions, 434 which provides strong evidence that NPs induced PC production is one of the 435 436 important mechanisms for the mitigation of Cd stress. Overall, our results suggest that regulation of the enzymatic antioxidant system and PC production are important 437 mechanisms for NPs-induced relief of Cd stress. 438

Phytohormones are crucial in plant growth and their regulation is an important 439 mechanism for abiotic stress alleviation (Wani et al., 2016). The present study 440 identified the important role of ABA and IPA in the mitigation of Cd stress. ABA is a 441 'stress phytohormone', which is typically increased when plants tried to cope with 442 stresses (Vishwakarma et al., 2017). The supplementation of NPs under Cd stress led 443 444 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 445 defined as a cytokinin (Mano and Nemoto, 2012). Interestingly, the adding of NPs 446 under Cd stress seemed to promote the synthesis of IPA. This might be attributed to 447 the NPs promoted production of new rice roots which contain large number of 448 cytokinin. It is important to point out that the contents of phytohormones in plants are 449

often interrelated. For instance, GA4 is usually positively correlated with the content 450 of IAA. The IAA content is also correlated with BR because IAA is regulated by BR 451 through the inhibition of the auxin oxidase activity. These connections among the 452 three phytohormones explained the similar trend observed in this study (Kandhol et 453 al., 2022; Tripathi et al., 2022; Wang et al., 2022). In addition to those reported in the 454 present study, some previous reports have also demonstrated that NPs can modulate 455 plant phytohormones to alleviate environmental stress, such as TiO₂ NPs alleviated 456 457 Cd stress (Ji et al., 2017) and CeO2 NPs alleviated N overload or deficiency (Wang et al., 2020). Undoubtedly, further studies are required to gain more insight into Cd and 458 NPs induced phytohormonal responses to optimize this potentially important abiotic 459 460 stress management strategy.

Cd stress leads to a serious imbalance in plant mineral element homeostasis, 461 which may be one of the important causes of stunting in rice. We found that the Cd 462 content in rice decreased after the addition of NPs, and correspondingly the mineral 463 464 homeostasis was altered. This may be partly due to the uptake of ions by plants in the form of chemical equivalents, and the competing uptake between ions (Cui et al., 465 466 2008; Qin et al., 2020). In addition, previous reports also showed that the small size of NPs may affect ion transporters, thus changing the mineral homeostasis (Zhou et al., 467 2021). Thus, the reduction of Cd induced by nZVI (Figure 5B) may have elevated the 468 concentrations of Cu and Zn in shoots. Interestingly, nHA displayed different effects 469 on nutrient homeostasis in plant tissues from Fe-based NPs. We found a further 470 471 reduction of the five mineral elements in the stems resulting from the addition of high concentrations of nHA compared to the Cd treatment alone. The greater interference 472 473 of ion transport by nHA than nZVI might be an important reason why nHA was less effective than nZVI in alleviating Cd stress. The growth and development of rice are 474 dependent on a variety of inorganic nutrients. Deficiency or excess of these elements 475 can even lead to poor development (Zhang et al., 2021). The results indicate that the 476 regulation of inorganic nutrient homeostasis may be another meaningful way for NPs 477 to alleviate Cd stress. High concentrations of nZVI significantly elevated the Fe and 478

Cu contents in the roots and Cu and Zn elements in the shoots to a comparable level 479 in the control plants. Despite the rise of P after nHA addition, nHA might result in a 480 further imbalance in the homeostasis of the remaining inorganic nutrients. Fe is an 481 essential component of Fe oxygen reduction protein in plants, which is involved in 482 plant photosynthesis, nitrate reduction, biological nitrogen fixation and other electron 483 transfer (Hussain et al., 2019). Similarly, Cu plays a crucial role in electron transfer 484 during photosynthesis, and it is as well an essential part of SOD (Ma et al., 2020). 485 486 Therefore, the high Fe and Cu content induced by nZVI treatment may effectively promote photosynthesis, improve nutrient transport (Pishkar et al., 2022) and promote 487 growth of rice under Cd stress. 488

489 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 490 Cd induced significantly lower Cd accumulation in the rice compared to the other two 491 NPs. It should also be noted that all NPs except nZVI resulted in elevated Cd content 492 493 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 494 fraction of Cd will be mostly retained in the roots because of the low upward transport 495 of NPs, resulting in a relatively high Cd content in the root compared to the shoot. 496 Gong et al. (2017) also demonstrated that nZVI at 1 μ g/L promoted Cd accumulation 497 by 50% in roots of ramie. The significant reduction of Cd in shoots when Cd and NPs 498 were co-exposed suggests that the effect of NPs in mitigating phytotoxicity is a 499 function of reduced Cd absorb and translocation. The Cd content in the root system 500 501 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 502 the length and weight of the root system, indicating that it effectively relieved the Cd 503 stress in rice roots. Interestingly, more severe Cd toxicity may significantly lower Cd 504 in the soluble fraction in plant roots, resulting in more Cd accumulation in the cell 505 walls than shoot (Lu et al., 2017). The constituents of plant cell walls could provide 506 numerous potential ligands with different functional group, like hydroxyl, carboxyl, 507

aldehyde and amino groups (Wang et al., 2009), that can provide adsorption sites or 508 form complexes with Cd. Compared with Cd alone treatment, the relatively higher 509 integrity of the cell wall treated with nZVI reduced the number of Cd ions entering 510 the organelles, thus reducing the damage of Cd to the root cells. It should be noted 511 that the destructive method (Differential centrifugation method, detailed in 512 513 Supplementary materials S6) that used in this study may cause redistribution of Cd in vacuole to cell walls during the sample preparation. However, this potential drawback 514 may not significantly change the overall observations in this study even though it may 515 underestimate the actual soluble fraction of Cd in the vacuoles. 516

The adsorption results further demonstrated that the final Cd concentration in the 517 Kimura B after 14 days of nZVI exposure was much lower than that of nHA and 518 nFe₂O₃, which can be attributed to the strong adsorption capacity of nZVI for Cd. The 519 520 surface charge of NPs and the solution pH are important factors affecting the uptake of NPs. The adsorption capacity of nFe₂O₃ for Cd is much lower than that of the other 521 two NPs due to electrostatic repulsion (nFe₂O₃ has a zeta potential of about +14.5 mV 522 523 at pH 5.5) (Gao et al., 2004). Plants may release protons or alkaline secretion to balance Cd uptake as a response to environmental stress, which would also alter 524 nutrient solution pH, Cd morphology, and NPs adsorption effects (Ryan et al., 2001). 525 In addition, the nanomaterials themselves also have the potential to alter the 526 environmental pH (Adeel et al., 2021; Shakoor et al., 2023). pH is a key factor 527 affecting the bioavailability of Cd, which usually shows a tendency to enhance 528 bioavailability with decreasing pH (Van Gestel and Koolhaas, 2004). High 529 concentration of nZVI induced a slight increase in the pH of rice solution compared to 530 the initial pH, while the opposite was observed for nFe₂O₃ and nHA treatments, 531 suggesting that nZVI can alleviate Cd stress by changing the pH. For a detailed 532 discussion of the dynamics of pH in solution see Section 1, S11. 533

Iron plaque function as a barrier to prevent the entry of phytotoxic compounds such as Cd into plant roots (Yu et al., 2020), and reduce their uptake by roots (Bao et al., 2019). The major constituents of iron plaques are reported to be fibrous iron ore

 $(\gamma$ -FeOOH) and needle iron ore (α -FeOOH), which easily incorporate Cd via hydroxyl 537 groups (Chen et al., 2017). Significant increase in iron plaques content when exposed 538 to Cd alone. The reason may be that the plants were stimulated to promote iron plaque 539 production under a high concentration of Cd stress, thereby alleviating the oxidative 540 stress effect. Similarly, (Wu et al., 2021) demonstrated a positive correlation between 541 the As content and the iron plaques. For the system with Fe-based NPs addition, the 542 total amount of iron plaques was further increased with increasing dose of Fe-based 543 544 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 545 plaques caused by 200 mg/L nFe₂O₃ was seen to be more pronounced in the pictures 546 (Figure 1G), yet the highest iron concentration in the DCB extracts was in the nZVI 547 group. Some of the NPs are difficult to be washed by running water or have entered 548 the epidermal plant cells. Therefore, we speculate that the DCB may extracted this 549 part of iron-based NPs, leading to the high iron plaques concentration. In conclusion, 550 our study suggests that iron plaques are a natural and effective barrier to Cd 551 552 accumulation in the rice root system and nZVI can further strengthen this barrier to lower Cd accumulation in rice shoots. 553

4.3 Regulation of iron transport protein expression by NPs

The mechanism was further determined by measuring the genes for Cd uptake and 555 translocation. We found that the expression of three genes, OsNRAMP5, OsHMA3, 556 and OsIRT1, returned to control levels after nHA and nZVI treatments, indicating that 557 Cd uptake was effectively inhibited. However, these three genes were further elevated 558 559 after exposure to nFe₂O₃, which is consistent with the results of Cd accumulation in rice. The release of Fe ions from nFe₂O₃ is likely a reason for the increase of the 560 expressions of these proteins (Figure S8A). Importantly, the protein expression data 561 align with the low Cd accumulation in rice shoots exposed to Cd+NPs, as compared 562 to Cd treatment. The expression of LCT1 in shoots showed a striking increase under 563 nFe2O3 induction, emphasizing the overload of Fe transporter proteins (LCT1). It is 564 speculated that the release of Fe ions from Fe-based NPs may have caused the up-565

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.

571 *5 Conclusions*

Exposure to the three NPs alone did not have any significant adverse effects on 572 rice development, except for high concentrations of nHA. Under Cd stress, the 573 amendment of all three NPs significantly reduced the phytotoxicity of Cd in rice. The 574 NPs reduced the bioavailability of Cd and mitigated its phytotoxicity a range of 575 mechanisms including adsorption, promotion of iron plaque formation, activating 576 plant antioxidant defense systems, regulation of PC production, hormone homeostasis, 577 mineral homeostasis, and Cd transporter protein expression. In this study, nZVI at 200 578 mg/L was the strongest in decreasing phytotoxicity and Cd accumulation in rice, 579 mainly by enhancing Cd chelation in vesicles and reducing Cd transport capacity. 580 581 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 582 concern, the findings in this study offer important implications for the influence of 583 nanotechnology on food safety. The present study elucidated the mechanisms of 584 altered Cd uptake, translocation and accumulation by three different NPs and 585 highlighted their potential roles in lowering Cd risks from food consumption. More 586 work focusing on the interaction of NPs and heavy metals in complex environmental 587 media over long periods will further illustrate the potential of this novel sustainable 588 589 agricultural strategy.

590

591 Acknowledgment

The project was supported by the National Key R&D Program of China (2017YFD0801300, 2017YFD0801103). Additional support from EU H2020 project NanoSolveIT (Grant Agreement 814572) and NanoCommons (Grant Agreement 731032) was acknowledged. 596

597 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.
- 603

604 Conflict of interest

605 The authors declare no conflict of interest.

606

607 **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.

- Brookbank, B.P., Patel, J., Gazzarrini, S., Nambara, E., 2021. Role of Basal ABA in Plant Growth and
 Development. GENES 12. 10.3390/genes12121936.
- Cao, X., Yue, L., Wang, C., Luo, X., Zhang, C., Zhao, X., Wu, F., White, J.C., Wang, Z., Xing, B.,
 2022. Foliar Application with Iron Oxide Nanomaterials Stimulate Nitrogen Fixation, Yield, and
 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.
- Cui, Y., Zhang, X., Zhu, Y., 2008. Does copper reduce cadmium uptake by different rice genotypes? J.
 Environ. Sci.-China 20, 332-338. https://doi.org/10.1016/S1001-0742(08)60052-2.
- Gao, Y., Wahi, R., Kan, A.T., Falkner, J.C., Colvin, V.L., Tomson, M.B., 2004. Adsorption of
 cadmium on anatase nanoparticles effect of crystal size and pH. Langmuir 20, 9585-9593.
- Gong, X., Huang, D., Liu, Y., Zeng, G., Wang, R., Wan, J., Zhang, C., Cheng, M., Qin, X., Xue, W.,
 2017. Stabilized Nanoscale Zerovalent Iron Mediated Cadmium Accumulation and Oxidative
 Damage of Boehmeria nivea (L.) Gaudich Cultivated in Cadmium Contaminated Sediments.
 Environ. Sci. Technol. 51, 11308-11316. 10.1021/acs.est.7b03164.
- Guha, T., Barman, S., Mukherjee, A., Kundu, R., 2020. Nano-scale zero valent iron modulates Fe/Cd
 transporters and immobilizes soil Cd for production of Cd free rice. Chemosphere 260, 127533.
 https://doi.org/10.1016/j.chemosphere.2020.127533.
- Huang, G., Pan, D., Wang, M., Zhong, S., Huang, Y., Li, F., Li, X., Xing, B., 2022. Regulation of iron
 and cadmium uptake in rice roots by iron(iii) oxide nanoparticles: insights from iron plaque
 formation, gene expression, and nanoparticle accumulation. Environmental Science: Nano 9, 40934103. 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 oxide nanoparticles. iron Ecotox. Environ. Safe. 173, 156-164. 661 https://doi.org/10.1016/j.ecoenv.2019.01.118.
- Hussain, A., Ali, S., Rizwan, M., Ur Rehman, M.Z., Qayyum, M.F., Wang, H., Rinklebe, J., 2019.
 Responses of wheat (Triticum aestivum) plants grown in a Cd contaminated soil to the application
 of iron oxide nanoparticles. Ecotox. Environ. Safe. 173, 156-164.
- International Agency for Research on Cancer, 2012. Agents classified by the IARC Monographs.
 https://monographs.iarc.who.int/agents-classified-by-the-iarc/ (accessed2021).
- Ji, Y., Zhou, Y., Ma, C., Feng, Y., Hao, Y., Rui, Y., Wu, W., Gui, X., Le, V.N., Han, Y., Wang, Y.,
 Xing, B., Liu, L., Cao, W., 2017. Jointed toxicity of TiO 2 NPs and Cd to rice seedlings: NPs alleviated Cd toxicity and Cd promoted NPs uptake. Plant Physiol. Bioch. 110, 82.
- Kandhol, N., Singh, V.P., White, J.C., Phan Tran, L., Tripathi, D.K., 2022. Plant growth hormones and
 nanomaterial interface: Linking development to defence. Plant Cell Physiol.
- Kaya, C., _enbayram, M., Akram, N.A., Ashraf, M., Alyemeni, M.N., Ahmad, P., 2020. Sulfurenriched leonardite and humic acid soil amendments enhance tolerance to drought and phosphorus
 deficiency stress in maize (Zea mays L.). Sci. Rep.-UK 10, 6432. 10.1038/s41598-020-62669-6.
- Kaya, C., Ashraf, M., Alyemeni, M.N., Ahmad, P., 2020. Responses of nitric oxide and hydrogen
 sulfide in regulating oxidative defence system in wheat plants grown under cadmium stress.
 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

- 679 nitric oxide improves tolerance to cadmium toxicity by reducing oxidative stress in wheat plants.
 680 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.
- 684 Kaya, C., Sarioglu, A., Ashraf, M., Alyemeni, M.N., Ahmad, P., 2022. The combined supplementation 685 of melatonin and salicylic acid effectively detoxifies arsenic toxicity by modulating phytochelatins 686 and nitrogen metabolism in pepper plants. Environ. Pollut. 297. 118727. https://doi.org/10.1016/j.envpol.2021.118727. 687
- 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.

- Michálková, Z., Martínez-Fernández, D., Komárek, M., 2017. Interactions of two novel stabilizing
 amendments with sunflower plants grown in a contaminated soil. Chemosphere 186, 374-380.
- Pishkar, L., Yousefi, S., Iranbakhsh, A., 2022. Foliar application of Zinc oxide nanoparticles alleviates
 cadmium toxicity in purslane by maintaining nutrients homeostasis and improving the activity of
 antioxidant enzymes and glyoxalase system. Ecotoxicology 31, 667-678. 10.1007/s10646-02202533-7.
- 730 Poole Jr, C.P., Owens, F.J., 2003. Introduction to nanotechnology. John Wiley & Sons.
- Qin, S., Liu, H., Nie, Z., Rengel, Z., Gao, W., Li, C., Zhao, P., 2020. Toxicity of cadmium and its
 competition with mineral nutrients for uptake by plants: A review. Pedosphere 30, 168-180.
 https://doi.org/10.1016/S1002-0160(20)60002-9.
- Rehman, M.Z.U., Rizwan, M., Hussain, A., Saqib, M., Ali, S., Sohail, M.I., Shafiq, M., Hafeez, F.,
 2018. Alleviation of cadmium (Cd) toxicity and minimizing its uptake in wheat (Triticum aestivum)
 by using organic carbon sources in Cd-spiked soil. Environ. Pollut. 241, 557-565.
 10.1016/j.envpol.2018.06.005.
- Rizwan, M., Ali, S., Adrees, M., Rizvi, H., Zia-Ur-Rehman, M., Hannan, F., Qayyum, M.F., Hafeez, F.,
 Ok, Y.S., 2016. Cadmium stress in rice: toxic effects, tolerance mechanisms, and management: a
 critical review. Environ. Sci. Pollut. R. 23, 17859-17879. 10.1007/s11356-016-6436-4.
- Rossi, L., Sharifan, H., Zhang, W., Schwab, A.P., Ma, X., 2018. Mutual effects and in planta accumulation of co-existing cerium oxide nanoparticles and cadmium in hydroponically grown soybean (Glycine max (L.) Merr.). Environmental Science: Nano 5, 150-157. 10.1039/C7EN00931C.
- Rossi, L., Zhang, W., Schwab, A.P., Ma, X., 2017. Uptake, Accumulation, and in Planta Distribution of
 Coexisting Cerium Oxide Nanoparticles and Cadmium in Glycine max (L.) Merr. . Environ. Sci.
 Technol. 51, 12815-12824. 10.1021/acs.est.7b03363.
- Ryan, P.R., Delhaize, E., Jones, D.L., 2001. Function and mechanism of organic anion exudation from
 plant roots. Annu. Rev. Plant Biol. 52, 527.
- Sebastian, A., Prasad, M.N.V., 2016. Iron plaque decreases cadmium accumulation in Oryza sativa L.
 and serves as a source of iron. Plant Biology 18, 1008-1015. https://doi.org/10.1111/plb.12484.
- Shakoor, N., Adeel, M., Ahmad, M.A., Hussain, M., Azeem, I., Zain, M., Zhou, P., Li, Y., Xu, M., Rui,
 Y., 2023. Environment relevant concentrations of lithium influence soybean development via
 metabolic reprogramming. J. Hazard. Mater. 441, 129898.
 https://doi.org/10.1016/j.jhazmat.2022.129898.
- Shakoor, N., Adeel, M., Zain, M., Zhang, P., Ahmad, M.A., Farooq, T., Zhou, P., Azeem, I., Rizwan,
 M., Guo, K., Jilani, G., Ahmar, S., Maqbool, S., Ming, X., Rui, Y., 2022. Exposure of cherry
 radish (Raphanus sativus L. var. Radculus Pers) to iron-based nanoparticles enhances its
 nutritional quality by trigging the essential elements. NanoImpact 25, 100388.
 https://doi.org/10.1016/j.impact.2022.100388.
- Tagawa, K., Tsujimoto, H.Y., Arnon, D.I., 1963. Role of chloroplast ferredoxin in the energy
 conversion process of photosynthesis. Proceedings of the National Academy of Sciences 49, 567572.
- Tang, L., Mao, B., Li, Y., Lv, Q., Zhang, L., Chen, C., He, H., Wang, W., Zeng, X., Shao, Y., Pan, Y.,
 Hu, Y., Peng, Y., Fu, X., Li, H., Xia, S., Zhao, B., 2017. Knockout of OsNramp5 using the
 CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. Sci.

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

- 768The Ministry Of Environmental Protection, The Ministry Of Land Resources, 2014. Bulletin of769nationalsoilpollutionsurvey.
- 770 https://www.mee.gov.cn/gkml/sthjbgw/qt/201404/t20140417_270670.html (accessed.
- Tripathi, D., Singh, M., Pandey-Rai, S., 2022. Crosstalk of nanoparticles and phytohormones regulate
 plant growth and metabolism under abiotic and biotic stress. Plant Stress, 100107.
- Uraguchi, S., Kamiya, T., Sakamoto, T., Kasai, K., Sato, Y., Nagamura, Y., Yoshida, A., Kyozuka, J.,
 Ishikawa, S., Fujiwara, T., 2011. Low-affinity cation transporter (OsLCT1) regulates cadmium
 transport into rice grains. Proceedings of the National Academy of Sciences 108, 20959-20964.
 10.1073/pnas.1116531109.
- Van Gestel, C.A., Koolhaas, J.E., 2004. Water extractability, free ion activity, and pH explain
 cadmium sorption and toxicity to Folsomia candida (Collembola) in seven soil pH combinations.
 Environmental Toxicology and Chemistry: An International Journal 23, 1822-1833.
- Veneklaas, E.J., Lambers, H., Bragg, J., Finnegan, P.M., Lovelock, C.E., Plaxton, W.C., Price, C.A.,
 Scheible, W.R., Shane, M.W., White, P.J., 2012. Opportunities for improving phosphorus use
 efficiency in crop plants. New phytologist 195, 306-320.
- Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R.K., Kumar, V., Verma, R.,
 Upadhyay, R.G., Pandey, M., Sharma, S., 2017. Abscisic Acid Signaling and Abiotic Stress
 Tolerance in Plants: A Review on Current Knowledge and Future Prospects. Front. Plant Sci. 8,
 161.
- Wan, Y., Wang, K., Liu, Z., Yu, Y., Wang, Q., Li, H., 2019. Effect of selenium on the subcellular
 distribution of cadmium and oxidative stress induced by cadmium in rice (Oryza sativa L.).
 Environ. Sci. Pollut. R. 26, 16220-16228. 10.1007/s11356-019-04975-9.
- Wang, J., Yuan, J., Yang, Z., Huang, B., Zhou, Y., Xin, J., Gong, Y., Yu, H., 2009. Variation in
 Cadmium Accumulation among 30 Cultivars and Cadmium Subcellular Distribution in 2 Selected
 Cultivars of Water Spinach (Ipomoea aquatica Forsk.). J. Agr. Food Chem. 57, 8942-8949.
 10.1021/jf900812s.
- Wang, Y., Wang, L., Ma, C., Wang, K., Hao, Y., Chen, Q., Mo, Y., Rui, Y., 2019. Effects of cerium
 oxide on rice seedlings as affected by co-exposure of cadmium and salt. Environ. Pollut. 252,
 1087-1096. https://doi.org/10.1016/j.envpol.2019.06.007.
- Wang, Y., Zhang, P., Li, M., Guo, Z., Ullah, S., Rui, Y., Lynch, I., 2020. Alleviation of nitrogen stress
 in rice (Oryza sativa) by ceria nanoparticles. Environmental Science: Nano 7, 2930-2940.
 10.1039/D0EN00757A.
- Wang, Z., Tang, J., Zhu, L., Feng, Y., Yue, L., Wang, C., Xiao, Z., Chen, F., 2022. Nanomaterialinduced modulation of hormonal pathways enhances plant cell growth. Environmental Science:
 Nano 9, 1578-1590. 10.1039/D2EN00251E.
- Wani, S.H., Kumar, V., Shriram, V., Sah, S.K., 2016. Phytohormones and their metabolic engineering
 for abiotic stress tolerance in crop plants. The Crop Journal 4, 162-176.
 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.): А 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. Nanobiotechnologybased strategies for enhanced crop stress resilience. Nature Food 3, 829-836. 10.1038/s43016-02200596-7.
- 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.

852