



HAL
open science

Mitigating cadmium exposure risk in rice with foliar nano-selenium: Investigations through Caco-2 human cell line in-vivo bioavailability assay

Bilal Hussain, Xianyuan Yin, Qiang Lin, Yasir Hamid, Muhammad Usman Hadi, Muhammad Laeeq Ur Rehman Hashmi, Min Lu, Muhammad Imran Taqi, Zhenli He, Xiao-E Yang

► To cite this version:

Bilal Hussain, Xianyuan Yin, Qiang Lin, Yasir Hamid, Muhammad Usman Hadi, et al.. Mitigating cadmium exposure risk in rice with foliar nano-selenium: Investigations through Caco-2 human cell line in-vivo bioavailability assay. *Environmental Pollution*, 2024, 356, pp.124356. 10.1016/j.envpol.2024.124356 . hal-04646070

HAL Id: hal-04646070

<https://hal.science/hal-04646070v1>

Submitted on 12 Sep 2024

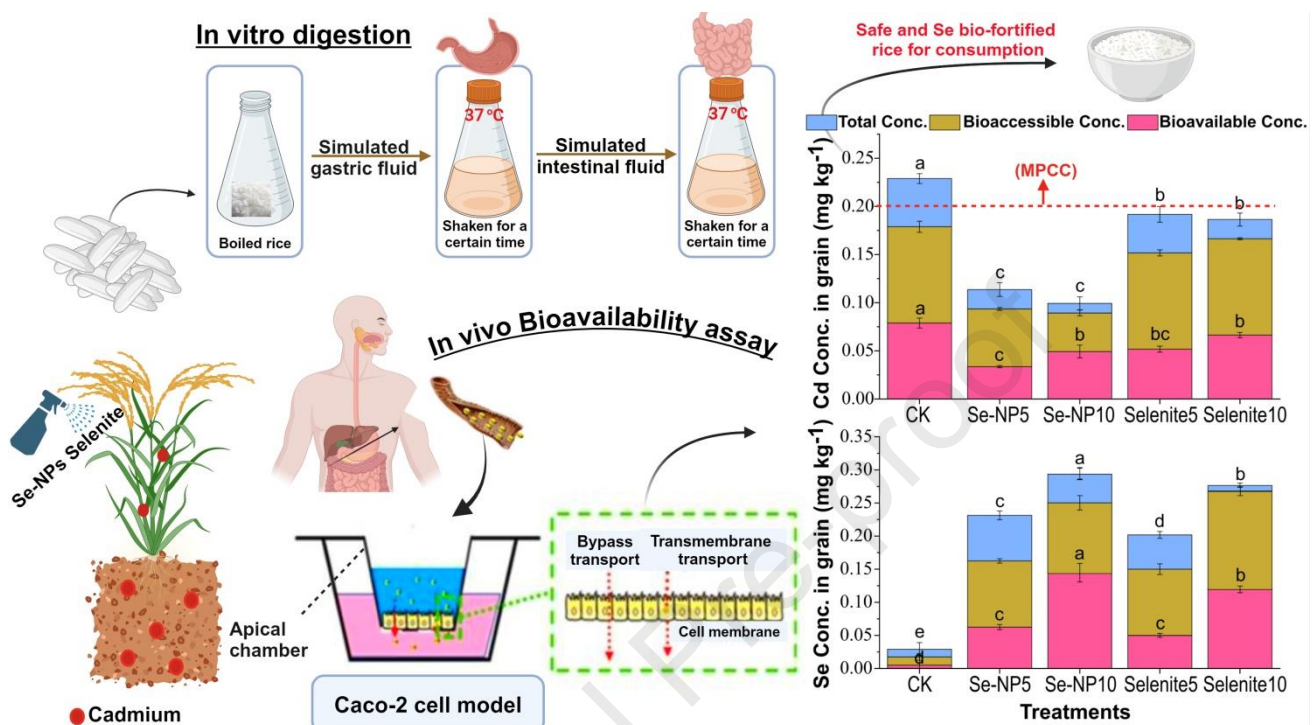
HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Graphical Abstract



1 **Mitigating cadmium exposure risk in rice with foliar nano-selenium:**
2 **Investigations through Caco-2 human cell line *in-vivo* bioavailability assay**

3 Bilal Hussain^{1a}, Xianyun Yin^{2a}, Qiang Lin¹, Yasir Hamid¹, Muhammad Usman³,
4 Muhammad Laeeq-ur-Rehman Hashmi¹, Min Lu⁴, Muhammad Imran Taqi⁵, Zhenli
5 He⁶, Xiao e Yang^{1*}

6

7 ¹Ministry of Education (MOE) Key Laboratory of Environmental Remediation and
8 Ecosystem Health, College of Environmental and Resources Science, Zhejiang
9 University, Hangzhou 310058, People's Republic of China

10 ²Beautiful Rural Construction Center of Quzhou, Quzhou 324003, Zhejiang, China

11 ³Université de Rennes, Ecole National e Supérieure de Chimie de Rennes, CNRS,
12 ISCR-UMR 6226, F-35000, Rennes, France

13 ⁴State Key Laboratory of Nutrient Use and Management, Tea Research Institute,
14 Shandong Academy of Agricultural Sciences, Jinan, China

15 ⁵Department of Soil Science, University of Sargodha, Sargodha Pakistan

16 ⁶Indian River Research and Education Center, Institute of Food and Agricultural
17 Sciences, University of Florida, Fort Pierce, Florida 34945, USA

18

19 ***For correspondence**

20 xeyang@zju.edu.cn (X. Yang)

21 **a Authors contribute equally**

22

23

Abstract

24 The contamination of paddy fields by cadmium and lead is a major issue in China.
25 The consumption of rice grown in heavy metals contaminated areas poses severe health
26 risks to humans, where bioavailability and bioaccessibility remains the critical factor
27 for risk determination. Selenium nanoparticles (Se-NPs) can mitigate the toxicity of
28 heavy metals in plants. However, there exists limited information regarding the role of
29 Se-NPs in dictating cadmium (Cd) toxicity in rice for human consumption. Moreover,
30 the impact of Se-NPs under simultaneous field and laboratory controlled conditions is
31 rarely documented. To address this knowledge gap, a field experiment was conducted
32 followed by laboratory scale bioavailability assays. Foliar application of Se-NPs and
33 selenite (at 5, 10 mg L⁻¹) was performed to assess their efficiency in lowering Cd
34 accumulation, promoting Se biofortification in rice grains, and evaluating Cd exposure
35 risk from contaminated rice. Obtained results indicate that foliar treatments
36 significantly reduced the heavy metal accumulation in rice grains. Specifically, Se-NP
37 10 mg L⁻¹ demonstrated higher efficiency, reducing Cd and Pb by 56 and 32 %
38 respectively. However, inconsistent trends for bioavailable Cd (0.03 mg kg⁻¹) and
39 bioaccessible (0.04 mg kg⁻¹) were observed while simulated human rice intake.
40 Furthermore, the foliage application of Se-NPs and selenite improved rice quality by
41 elevating Se, Zn, Fe, and protein levels, while lowering phytic acid content in rice
42 grains. In summary, this study suggests the promising potential of foliage spraying of
43 Se-NPs in lowering the health risks associated with consuming Cd-contaminated rice.

44 **Keywords:** Cadmium, Caco-2 cell line, In-vitro assays, Laboratory try-out,

45 Nanoparticles, Selenium.

46 **1. Introduction**

47 Currently, the contamination of heavy metals, specifically cadmium (Cd) and lead
48 (Pb) in Chinese soils has reached a critical level. The national soil pollution survey of
49 China (2014) indicated that approximately 19% of soils are contaminated with heavy
50 metals, exceeding the established soil quality standards. It is widely recognized that
51 plant roots can easily absorb soil-available Cd and Pb, which are subsequently
52 transported to above-ground parts, posing a potential threat to human health via food
53 chain (Lin et al., 2022; Xiao et al., 2024). Rice is a staple food for over 50 % of the
54 global community, with the capacity to assimilate heavy metals in paddy fields, thereby
55 transferring them to grains even at low concentrations (Hamid et al., 2023). The
56 accumulation of Cd and Pb in edible parts of crops presents a potential risk to human
57 health (Zhang et al., 2022).

58 Selenium (Se) is a crucial micronutrient known for its essential role in various
59 functions across humans, animals, and other organisms. (Islam et al., 2020; Wang et al.,
60 2023). Its deficiency in soils leads to insufficient intake of Se, resulting in a variety of
61 diseases, including cancer, heart diseases, Kashin-Beck disease, and compromised
62 immunity among individuals residing in Se-deficient regions (Fang et al., 2019). Se
63 deficiency affects 72 % of China's overall land area, with rice grains showing a reduced
64 Se content of about $20 \mu\text{g kg}^{-1}$ (Dinh et al., 2018; Song et al., 2023). Consequently,
65 Chinese individuals have an average daily Se intake (26-34 μg) that falls significantly
66 below the recommended range of 55-85 μg for adults (Ramakrishnan et al., 2022).

67 Previous studies have demonstrated that Se has the potential to alleviate the toxic
68 effects of heavy metals in plants (Hussain et al., 2020; Kieliszek and Sandoval, 2023).
69 For example, Se application in a hydroponic experiment prominently reduced the Cd
70 concentration in rice plants (Zhao et al., 2023), simultaneously, reducing the
71 accumulation of the heavy metals in rice grains cultivated in contaminated farmland (Li
72 et al., 2022).

73 Nanotechnology has emerged as an evolving and swiftly advancing tool kit with
74 novel applications in food science, agriculture and medicine (Usman et al., 2020; Zhu
75 et al., 2022). For instance, foliage application of nano-Se has shown potential in
76 effectively reducing the Cd accumulation in pepper and brassica nappus plant by
77 inhibiting the Cd translocation in solution culture experiments (li et al., 2022; Zhu et al.,
78 2022). It has been documented that nano-Se possesses remarkable bioavailability and
79 lower toxicity compared to selenite or selenate, proving to be a promising material for
80 utilization in both biological and nano-technological realms, particularly for enhancing
81 crop nutrition and protection, as well as increasing Se bioavailability with its foliar
82 application (Hussain et al., 2020). Se-NPs have garnered attention for their role in
83 mitigating the effects of heavy metals through various physiological processes
84 (Kamran et al., 2020). The Se fertilizer can mitigate the negative effects caused by
85 reactive oxygen species by enhancing the defense mechanisms (Ran et al., 2024).
86 Barman and Kundu, (2023) reported that Se supplementation resulted in reduced Cd
87 concentration and oxidative damage in plants by enhancing antioxidant enzymatic
88 activity. Meanwhile, numerous examinations have reported that foliage

89 supplementation of Se-NPs down regulates the Cd transporter proteins in rice leaves
90 and peduncles (Wang et al., 2020). Foliage supplementation of Se down-regulates
91 *OsLCT1*, *osNramp5* expressions and up-regulates the *OsHMA3* proteins to detoxify Cd
92 in vacuolar (Nedjimi, 2024). The Cd transporter proteins, including *OsLCT1*, *OsCCx2*,
93 and *OsHMA2* in leaves and peduncles, were down regulated with increasing Se-NPs
94 and Si concentrations (Barman and Kundi, 2023).

95 However, significant research gaps exist regarding the effects of nano-fertilization
96 of Se on Se and Cd bioavailability in human simulated model (Caco-2 cells approach),
97 as well as reduced Cd toxicity in rice grains, particularly under field conditions
98 followed by laboratory bioaccessibility/bioavailability assay. Although, *in vivo* studies
99 are ideal for addressing the Se and Cd bioavailability in human body, their intricate
100 nature and high cost restricts their large-scale applications (Khanum and Platel, 2019).
101 Therefore, to overcome the cost issue, an *in vitro*/Caco-2 approach can more accurately
102 depict the actual availability of Se and Cd to humans, serving as an alternative to *in vivo*
103 studies. Lin et al. (2022) found that foliar supplementation of nano-Zn to rice crops
104 decreased the potential health hazards associated with Cd pollution, as demonstrated by
105 *in vitro* bioaccessibility and *in vivo* bioavailability assessments.

106 Therefore, a field study was conducted to evaluate the impact of various dosages of
107 foliar-applied sodium selenite (Na_2SeO_3) and laboratory-prepared Se-NPs on heavy
108 metal accumulation in rice grown in contaminated farmland. This study aims to (i)
109 reduce the accumulation of Cd and Pb in rice grains while simultaneously enhancing Se
110 biofortification, (ii) comparatively assess the effectiveness of selenite and Se-NPs on

111 Se and Cd bioavailability in rice grains (evidenced through
112 bioaccessibility/bioavailability assay), and (iii) compare the response of sodium
113 selenite and Se-NPs on Cd and Pb translocation from flag leaves to rice grains, and their
114 impact on the organic and in-organic Se proportions in rice grains.

115 2. Materials and methods

116 2.1 Synthesis and characterization of selenium nanoparticles(Se-NPs)

117 Se-NPs were synthesized by a chemical reduction approach, following a slightly
118 modified protocol from Hussain et al. (2020). In summary, a 0.01 M solution of sodium
119 dodecyl sulfate (SDS) was formulated in ultra-pure water to ensure a final
120 concentration at maximum capacity. While selenous acid was prepared by dissolving
121 SeO_2 (Sigma) and the reducing agent was made by dissolving $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in the
122 SDS solution, with a 5.20 mM concentration. To examine the effect of varying
123 concentrations of different reducing agents on particle size, a concentration of 156 mM
124 for the reducing agent was tested. The reducing agent was slowly added drop by drop to
125 the selenous acid solution and the resulting mixture was then allowed to completely
126 reduce for a sufficient time after each reaction. The solution was consistently stirred
127 with a magnetic stirrer throughout the reduction reaction.

128 The size of various Se-NPs was assessed through transmission electron microscopy
129 (TEM, USA). To eliminate excess compounds like SDS, the colloids underwent initial
130 flocculation treatment. The elemental analysis of various colloids was conducted using
131 EDX spectroscopy (X'Pert PRO, Netherlands).

132 The NanoPlus (Zeta Potential & particle size estimation Analyzer, Norcross GA, USA)

133 was utilized to assess the zeta potential of Se-NPs. In summary, the sample was diluted
134 and sonicated for a sufficient amount of time to optimize particle dispersion.
135 Subsequently, the samples were transferred to the electrode cell, and measurements
136 were performed at a 90° angle with 25°C temperature.

137 **2.2 Site description**

138 A field experiment was conducted in the Qujiang county, Zhejiang China (30°35'19" N,
139 114°55'28" E), which experiences an average annual temperature of 17.31°C and
140 annual rainfall of 1665.6 millimeters. The soil in this area has been contaminated with
141 Cd due to waste water flooding and continuous use of chemical fertilizers. Total Cd and
142 Pb contents in soil were 0.83 mg kg⁻¹ and 104.46 mg kg⁻¹ respectively (Lin et al., 2024),
143 exceeding the standard parameters of the Soil Environmental Quality Standards of
144 China. Standard procedures for soil analysis as described by (Bao, 2008), were
145 followed and are outlined in Table 1.

146 **2.3 Experimental design**

147 The experiment was arranged in a (RCBD) layout with plot sizes of 20 m²,
148 consisting of three replications. Seedlings of the late rice variety SM-728 were cultured
149 by the end of May and transplanted after one month of growth. Uniform fertilizer
150 application and cultural practices were maintained across all plots throughout the
151 experiment. Se treatments comprised of lab-prepared Se-NPs and selenite, each
152 administered at two doses of 5 mg L⁻¹ and 10 mg L⁻¹ (Se-NPs 5, Se-NPs 10, selenite 5,
153 and selenite 10), along with a control (CK). Foliar application was performed twice
154 using a motorized boom sprayer at the tillering and booting stages on the plant surface,

155 while plant harvesting was conducted at the maturity stage. Yield characteristics (1
156 m²/plot) were assessed in the field at the time of harvesting. Plant samples were rinsed
157 twice with ultra-pure water and then separated into roots, shoots, and grains for
158 subsequent laboratory analysis.

159 **2.4 Plant analysis**

160 The total contents of Cd and Pb in grains, shoots and roots were determined by
161 digesting 0.2 g of ground samples with a mixture of HNO₃ and HClO₄ (5:1, v/v) for 6 h
162 at 150°C. The mixture was then cooled, diluted to 25 mL with ultra-pure water and the
163 concentration of heavy metals, as well as macro and micro-nutrients (Cu, Fe, Mg, Mn,
164 and Zn) was measured using ICP-MS (Bao, 2008).

165 **2.5 Determination of Se contents in grains**

166 The determination of the total Se contents in the rice grains was assessed by
167 digesting dried rice powder (0.2 g) with HNO₃-HClO₄ (4:1) at 60°C for 30 min,
168 followed by an additional 60 min at 100°C. Subsequently, 2.5 mL of the acid mixture
169 was added and heated to 150°C until a brown fog initially appeared, followed by the
170 later emergence of a white fog in the tubes. The tubes containing digestion mixture was
171 cooled to 100°C and 6 mM HCl (2.5 mL) was added to convert SeO₄²⁻ to SeO₃²⁻,
172 followed by further heating at 100°C until the disappearance of the brown fog. The
173 volume was calibrated and analyzed using AFS for Se determination (Sun et al., 2013).
174 The concentrations of inorganic and organic Se in rice grains were determined by
175 mixing 1 g of grain flour with 30 mL of distilled water in an Erlenmeyer flask,
176 sonicated for 30 min and subsequently centrifuging for 10 min at 5000 rpm. The

177 supernatant liquid was gathered and 5 mL of cyclohexane was introduced to the
178 solution, after 4 h water phase was collected in a beaker. The 6 mM HCl was introduced
179 to the solution and heated for 2-3 min. Subsequently, the solution was diluted with
180 distilled water to achieve a final volume of 10 mL and underwent to AFS for Se
181 determination (Hussain et al., 2020).

182 The total concentration of Se in soil was measured using a similar methodology to that
183 used for rice grains, except for the use of HNO₃-HClO₄ (in a ratio of 3:2, v/v) for
184 digestion.

185 **2.6 Cd and Se bioavailability assays**

186 Recently, *in vivo/in vitro* procedures to examine the gastrointestinal digestion, nutrient
187 and contaminants absorption in the human intestine through physiological oriented
188 extraction assays and human cell line (Caco-2) have gained attraction (Tang et al.,
189 2020).

190 ***In vitro*-digestion of rice sample:**

191 Cd and Se bio-accessibility in rice grains was evaluated using an *in vitro*
192 bioaccessibility assay with slight modifications (Lin et al., 2023). The rice powder (5g)
193 was immersed in 15 mL of buffer solution and the pH was adjusted to 2. It was then
194 incubated for 2 h at 37°C for complete digestion of rice powder, followed by the
195 addition of 0.5 mL pepsin solution.

196 Prior to start gastrointestinal digestion of the rice samples the pH was raised to 5 by
197 adding 1 M sodium bicarbonate. A solution containing 2.5 mL of pancreatin bile was
198 then supplemented in the samples, followed by another 2h of incubation. Subsequently,

199 the pH was adjusted to 7.2 with 0.5 M sodium hydro-oxide. After digestion, the
200 samples were centrifuged at 3500 rpm for 1 h at 4°C, and the supernatant was gathered,
201 filtered, and stored at -20°C for subsequent analysis.

202 The Se concentrations in the samples were measured using AFS, while the Cd
203 concentration was measured using ICP-MS. Subsequently, the bioaccessibility was
204 calculated using the formula described by Hussain et al. (2020):

$$\begin{aligned} & \text{Bioaccessibility (\%)} \\ & = \left[\frac{(\text{Se or Cd Conc.}) \text{in vitro digestion sol.} \times \text{Vol. } (\mu\text{g}) \text{total vol of in vitro digestion sol.}}{(\text{Se \& Cd Conc.}) \text{cooked polished rice} \times \text{Weight of cooked rice} (\mu\text{g})} \right] \times 100 \end{aligned}$$

207 ***Cell Culture:***

208 The assay employed Caco-2 cells, purchased from the cell biology institute (Shanghai,
209 China), which had undergone 10-14 aisles of growth. After culturing the cells in 25 cm²
210 flasks with specialized growth medium (Aziz et al., 2015), they were subjected to
211 incubation with 5 % carbon dioxide at 37°C and after every 48 h, the growth medium
212 was changed.

213 For the bioavailability of Se and Cd, Caco-2 cells were sub-cultured in 6 trans-well
214 plates treated with trypsin solution. 2.5 mL of complete DMEM was added to the
215 basolateral compartment and 1.5 mL were added to the permeable polycarbonate filters
216 (0.4 um pore size). The Caco-2 cell cultures were maintained for 10 days to achieve a
217 stable growth for optimal functional differentiation, with the medium being refreshed
218 every 2 days. Before the uptake experiment, the cultured medium was replaced, both
219 the mono and basolateral compartments were rinsed twice with phosphate-buffered
220 saline at 37°C to remove unattached adherent cells from the layer.

221 Rice grain solution 1.5 mL was introduced into the apical compartment and 2.5 mL of
 222 standard solution was added to the basolateral part. The solution from the basolateral
 223 compartment was collected after 2 h of incubation to determine the Se and Cd in the
 224 transport solution. The bioavailability in the Caco-2 cells was calculated (Aziz et al.,
 225 2015) as follows.

226 *Bioavailability*(%)

227 = 1

228
$$- \left[\frac{(Se\&Cd\text{Conc.})_{\text{apical chamber after transp.}} \times Vol. (\mu\text{g})_{\text{washing sol.}}}{(Se\&Cd\text{Conc.})_{\text{apical chamber before transp.}} \times Vol. \text{added digestion sol. } (\mu\text{g})} \right]$$

229 $\times 100 \times \textit{Bioaccessibility}$

230 The concentration of Cd in cell retention samples was analyzed using ICP-MS (Hussain
 231 et al., 2020), while the Se concentration was assessed using AFS.

232 **2.7 Rice quality parameters**

233 Phytic acid was quantified using the method established by Hussain et al. (2020)
 234 with specific modifications. A 0.5 g sample of rice flour was subjected to extraction
 235 with 10 mL of 0.2 M HCl, the mixture was shaken for 2h on a rotary shaker and then
 236 centrifuged for 10 min at 10,000 rpm. The supernatant solution of 2.5 mL was then
 237 mixed with 2mL of 0.2% iron chloride, after heating on a water bath for 30 min, the
 238 solution was cooled and centrifuged at 10000 rpm for 15 min.

239 The upper layer of liquid was discarded and the remaining residue washed thrice
 240 with distilled water. Subsequently, 3 mL of 1.5 M sodium hydroxide was added and
 241 centrifuged. The supernatant was discarded and the residue was then subjected to 3 mL
 242 of 0.5 M hydro chloride to solubilize the residues. The volume was adjusted to 10 mL

243 by diluting with ultra-pure water. The Fe contents were analyzed using ICP-MS. The
 244 phytic acid content was determined by multiplying the Fe content by a factor of 4.2.
 245 The Kjeldahl method, based on total nitrogen, was employed to determine the protein
 246 content in rice powder, following the protocol described by Lin et al. (2023).

247 **2.8 Metal translocation factors**

248 The factor of translocation for heavy metals was computed using the formula outlined
 249 by Hamid et al. (2019).

$$251 \quad \text{Translocation factor (TF \%)(Shoot – Grain)} = \frac{\text{Cd or Pb conc. in grains}}{\text{Cd or Pb conc. in shoot}} \times 100$$

250

$$252 \quad \text{Translocation factor(TF \%)(Root – Shoot)} = \frac{\text{Cd or pb conc. in shoot}}{\text{Cd or Pb conc. in roots}} \times 100$$

253 **2.9 Statistical analysis**

254 The data were analyzed using Excel software and presented as the means of three
 255 replicates (\pm SE). Treatment means were distinguished through one-way ANOVA with
 256 LSD's test ($P < 0.05$). Principal component analysis (PCA) and correlations were
 257 systematically performed by using R studio (version 6) to elucidate the relationship of
 258 the dataset. Graphical representations were generated using Origin Pro 24.

259 **3. Results and discussion**

260 **3.1 Characterization of Se-NPs**

261 The TEM images (Fig 1A) revealed an average particle size diameter of 18.74 ± 3.2
 262 nm. Moreover, EDX analysis illustrated the elemental proportions in the final
 263 suspension (Fig 1B-I), which accounted for carbon (C) at 27 %, oxygen (O) at 25%, Ca
 264 at 0.05 %, Mg at 0.1 %, and Se at 25.55 %. The dots indicate the presence of elements in

265 distinct colors.

266 The zeta potential (ZP) for the Se-NPs varied between -15 to 35 mV, indicating that
267 the produced NPs are highly stable particles. Particles with a ZP ranging from -30 to
268 +30 are considered as highly stable (Arshad et al., 2024).

269 **3.2 Grain and biological yield of rice**

270 The foliar application of selenite and Se-NPs had a non-significant effect on the
271 biological yield of rice (Figure 2). The average biological yield was 8381 kg ha⁻¹, with
272 the highest recorded at 8575 and 8440 kg ha⁻¹ for Se-NPs 10 and Se-NPs 5, respectively,
273 while the CK showed lowest biological yield (8305 kg ha⁻¹).

274 The application of Se-NPs 10 and selenite 10 resulted in a significant increase of 9%
275 and 5%, respectively, in rice grain yield. The maximum grain yield was recorded with
276 the Se-NPs 10 treatment (6183 kg ha⁻¹), where as CK had the lowest grain production of
277 5663 kg ha⁻¹. The non-significant effects of foliar treatments on the biological yield of
278 rice are consistent with previous experiments conducted in hydroponic culture, where
279 Se application to the nutrient solution did not improve the biomass of rice seedlings
280 (Farooq et al., 2022). Nevertheless, Yan et al. (2024) indicated an increase in grain yield
281 following the supplementation of Se at the dough stage of wheat. Our study results
282 revealed a significant increase in rice grain yield with the foliage application of
283 Se-NPs10 compared to the control (CK), highlighting the positive impact of Se
284 application on grain yield (Hussain et al., 2020). Moreover, a substantial correlation
285 was noted between the Se concentration and grain yield, which was also confirmed by
286 PCA analysis, indicating a positive relationship between these parameters (Figure 8, 9).

287 In a recent study, Wang et al. (2023) also demonstrated an increase in rice yield with the
288 application of optimized Se during vegetative growth. Therefore, foliar application of
289 Se fertilizers has a beneficial effect on grain yield, with no observable impact on the
290 biological yield.

291 **3.3 Cd and Pb uptake and accumulation in different parts of rice**

292 The results regarding the Cd and Pb accumulation in rice roots showed
293 non-significant effects with Se-NPs and selenite application (Figure 3B & 3C). The
294 average concentration of Cd and Pb concentration in rice roots were 4.65 and 15.27 mg
295 kg⁻¹, respectively. Meanwhile, foliar applications of Se-NPs and selenite led to decrease
296 Cd and Pb accumulation in both leaves and rice grains (Figure 3A). Specifically, foliar
297 application of Se-NPs 10 tended to reduce the accumulation of Cd and Pb in grains by
298 56% and 32%, respectively and leaves by 37% and 26%, respectively, compared to CK.
299 Furthermore, selenite 10 exhibited higher efficiency in effectively reducing Pb contents
300 in leaves, achieving a reduction of 29% compared to the CK.

301 It is widely acknowledged that studying the accumulation and translocation of Cd
302 and Pb in rice can contribute significantly to understanding the mechanisms of heavy
303 metals accumulation in plants, especially with the application of foliar treatments at
304 various plant growth stages (Huang et al., 2022). Therefore, the results of
305 comprehensive investigations into the application of foliar Se at critical growth stages
306 of rice plants, such as tillering and booting, revealed a significant decrease in Cd
307 content in rice tissues, particularly with Se-NPs 10 and Se-NPs 5 (Fig. 3). With Se
308 treatment, there was a noticeable downward trend in Cd and Pb accumulation in the

309 roots as compared to the CK, indicating a greater retention of these metals in the roots
310 (Fig.4). Furthermore, the proportion of Cd and Pb in leaves and polished grains was
311 lower than in the CK after Se treatment (Fig. 4), suggesting that Se might inhibit the
312 translocation of Cd and Pb to aboveground plant parts.

313 Excessive Pb has been reported to potentially exert negative effects on the activity
314 of defensive enzymes and the production of ROS species in plants. Consequently, this
315 may result in an increased production of ROS, serving as a protective mechanism
316 against Pb accumulation (Li et al., 2019). Furthermore, elevated levels of Pb can
317 influence oxidative stress by promoting the formation of hydroxyl radicals, H₂O₂ and
318 ROS (Alyemni et al., 2018). Se has the ability to reduce Pb accumulation in plants by
319 alleviating oxidative stress. For instance, Tang et al. (2020) reported foliage
320 supplementation of Se decreased the Cd and Pb levels in water spinach by reducing the
321 production the free radicals and H₂O₂. Similarly, Hussain et al. (2020) supplemented
322 the rice with Se and observed a 52.9% reduction in Pb concentration, thus mitigating
323 oxidative stress. Moreover, Se supplementation significantly reduced Pb accumulation
324 in sage (Bakhtiari et al., 2023), highlighting the beneficial effect of Se in mitigating
325 heavy metals toxicity (Zhu et al., 2022). The combination of Se and Si applied to
326 foliage exhibited a reduction in Pb accumulation by alleviating oxidative stress
327 (Alyemni et al., 2018). This suggests the necessity for further research to explore the
328 inhibitory or regulatory role of Se in gene expression.

329 **3.4 Effects of foliar treatments on translocations of metals**

330 The translocation (TFs) of Cd and Pb in different parts of plants varied with foliar

331 treatments (Figure 4). The highest TF (root-shoot) was observed in CK, followed by
332 selenite 10. In the case of Cd TF (shoot-grain), selenite 10 exhibited the highest
333 translocation by 38.2%, while Se-NP10 showed 19% translocation for Cd. However,
334 there was no significant difference in Pb translocation among different foliar treatment
335 and CK. It was observed that TF root-shoot and accumulation factor exhibited a
336 declining trend for both heavy metals (Figure 4), suggesting the inhibited translocation
337 of Cd and Pb to aboveground tissues of rice. Rice tends to accumulate higher levels of
338 Cd and Pb when cultivated in contaminated soil. However, foliar treatment with
339 Se-NP10 and Se-NP5 has demonstrated effectiveness in retaining Cd in the shoot,
340 functioning as a measure to reduce the excessive movement of Cd in rice grains (Lian et
341 al., 2022). Our findings also indicated a decrease in Cd and Pb translocation to rice
342 grains with foliar application, thereby suggesting mechanism for alleviating heavy
343 metals accumulation, such as enrichment in roots and inhibition of their translocation to
344 aboveground plant parts.

345 In this study, we noted that the foliar supplementation of Se-NP inhibits the
346 movement of heavy metals from root to shoots, as well as from shoots to the rice grains.
347 Nano Se may sequester Cd and Pb in the plant tissue, reducing their mobility and
348 availability (Zhu et al., 2022). Consequently, Nano Se lowers the accumulation of these
349 metals in edible plant parts, thus reducing associated health risks for humans (Wang et
350 al., 2023). These findings are directly linked to phytoremediation of the heavy metals,
351 involving plants removing heavy metals from soil and sequestering them within plant
352 cells by forming complexes or immobilizing them in plant cell wall (Tang et al., 2020).

353 The effect of Se-NPs is that they can enhance the effectiveness of phytoremediation
354 efforts, with inhibition of translocation being a crucial mechanism for reducing the
355 bioavailability of these heavy metals in plants. This mitigates the impact of heavy
356 metals on humans and thus reduces the health risk associated with Cd exposure.
357 Coupling phytoremediation with agro-production represents a significant advancement
358 in sustainable agriculture and environmental management. This offers a dual benefit,
359 farmers generate revenue through agro-production, while the environment benefits
360 from natural remediation as it cleans up contaminated soils and simultaneously
361 enhances agricultural productivity.

362 **3.5 Se concentration and speciation in rice grains**

363 The Se contents in brown rice increased dramatically in a dose dependent manner
364 with foliar application of various treatments (Figure 5). The Se concentration in brown
365 rice was 0.29 mg kg⁻¹ for Se-NP10 and 0.02 mg kg⁻¹ in the control (CK). It was
366 observed that the total concentration of organic Se in brown rice increased with higher
367 Se contents of foliar treatments. However, no notable difference in inorganic Se
368 concentration was observed among all treatments except CK. It was found that CK
369 exhibited the highest inorganic Se concentration at 35%. The results showed that
370 approximately 80% of the absorbed Se was in the form of organic Se in all treatments
371 except CK, suggesting that the species derived from foliar fertilizer have a significant
372 impact on the Se contents (Deng et al., 2017). Foliar application of Se-NPs during the
373 heading stage has been shown to transform inorganic Se into organic Se, thereby
374 increasing Se contents in cereal crops like rice and wheat grains (Hussain et al., 2020;

375 Viltres-Portales et al., 2024). However, there are reports indicating that the primary
376 portion of Se, assimilated from the soil to the plant, remains inorganic (Kieliszek and
377 Serrano-Sandoval, 2023).

378 Se is absorbed by rice plants from soil, organic Se is primarily retained in the roots,
379 whereas SeIV and SeVI are transferred to the shoots (Kong et al., 2023). The primary
380 component of organic Se is selenomethionine, which constitutes 61-98 % of the total
381 absorbed organic Se (Zeng et al., 2023). The presence of organic Se in the edible
382 portion of plant is of significant importance, as Se is an essential micronutrient for
383 various biological functions in living organisms (Kushwaha et al., 2021).
384 Selenomethionine is a crucial component of antioxidant enzymes like glutathione
385 peroxidase which safeguards body cells from the oxidative damage (Wang et al., 2024).
386 Furthermore, Se-met also plays an important role in thyroid function, immune function,
387 maintaining reproductive health, improving heart health and preventing cancer risk
388 (Kieliszek and Serrano-Sandoval, 2023).

389 **3.6 Cd and Se bioavailability/bioaccessibility in caco-2 cells**

390 Se bioaccessibility in the rice grains ranged between 43.02% and 55.32% with
391 different treatments (Fig. 6A and 6B). The highest bioaccessibility of Se was achieved
392 with selenite10 and Se-NPs10, indicating the impact of dosages on Se bioaccessibility.
393 In their study, Tokaloğlu et al., (2014) observed that Se from selenite-based nutritional
394 supplements exhibited the highest *in vitro* bioaccessibility compared to other Se-based
395 supplements. Significantly, a higher bioaccessibility of Cd (58.04 to 72.23%) was
396 observed with foliar Se-NPs and selenite treatments. These findings are consistent with

397 the observations of Mao et al. (2022), who noted an increase in the Cd bioaccessibility
398 in rice grains during gastric and intestinal digestion, this phenomenon might be
399 attribute to the highly acidic gastric juice (pH=1.5) containing active pepsin, which
400 demonstrates a substantial ability to release and dissolve bioaccessible Cd from rice
401 (Zhuang et al., 2016). This higher bioaccessibility of selenite compared to other Se
402 treatments may be attributed to the enrichment of untransformed Se (IV) in selenite
403 treated Se, leading to its increased bioaccessibility (Zhoua et al., 2021). Another
404 potential explanation could be that the proportion of Se protein-bound and other easily
405 extractable component-bound Se in selenite-treated samples might be higher than in
406 other Se treatments, thereby facilitating its release into gastrointestinal fluids (Zhoua et
407 al., 2021).

408 Subsequently, the bioaccessibe soluble fractions were used to evaluate the Caco-2
409 cells' uptake and transport of Cd (Fig. 6a and 6b). The bioavailable Cd concentration in
410 grains of CK treatment remained at $0.07 \pm 0.0014 \text{ mg kg}^{-1}$, illustrating highest
411 reduction of $0.03 \pm 0.0016 \text{ mg kg}^{-1}$ for Se-NPs10 supplementation. Meanwhile, the
412 bioavailable Se concentration was ranged from $0.01 \pm 0.0011 \text{ mg kg}^{-1}$ to 0.14 ± 0.0015
413 mg kg^{-1} in CK and Se-NP10 treatment, respectively. Interestingly, highest bioavailable
414 Se in rice grains was achieved with foliar supplementation of Se-NP10. However, no
415 significant difference in Se bioavailability was observed between Se-NP5 and selenite
416 5 applications. The transport of different forms of Se from the apical chamber to the
417 basolateral portion across the mono layer of Caco-2 cells depends on the organic
418 percentage present in the form like selenomethionine, nano Se and all of which are 3

419 fold more effective than selenite and it is suggested that a selenium form capable of
420 secretion from both sides could be generated, contrasting with selenite (Delaqua et al.,
421 2022; Xiong et al., 2023).

422 In the current study, the application of Se-NPs and selenite resulted in a reduced
423 total Cd concentration in grains, which was below the Chinese environmental quality
424 standards (0.2 mg kg^{-1}). Nonetheless, it was observed that Se-NP10 and selenite 10
425 were the most effective foliar treatments in lowering Cd levels in rice grains. Previous
426 studies have reported that the bioavailable Cd concentration in all treatments remained
427 below the permissible level of dietary intake due to non-dissolution of Cd in digestive
428 juice (Zheng et al., 2022; Lin et al., 2023). However only the bioavailable fraction of
429 Cd can be taken up by the human body and may have detrimental effects on vulnerable
430 organs (Beri et al., 2020). The Caco-2 bioavailability of Cd in Se-NPs and selenite is
431 significantly lower than control treatment. Therefore, these treatments can lower the
432 associated health risks with the ingestion of contaminated rice. Lowering the
433 bioavailability of Cd in contaminated rice also reduces the risk of diseases associated
434 with Cd toxicity, such as kidney damage, cardiovascular diseases and bone disorders
435 (Wang et al., 2023). Our results reveal fascinating findings concerning the Cd
436 bio-accessibility in rice grains, which were coincided with the total Cd contents.
437 Recently, Tang et al. (2020) also reported analogous findings, that supplementation of
438 Se and Zn led to decrease in total, and bioavailable fractions of Cd and Pb in spinach.
439 Hence, to assess the associated health risks with ingestion of contaminated rice, we
440 suggest using an in vitro treatment in minimizing Cd exposure in humans.

441 3.7 Effects of foliar treatments on nutritional quality of rice

442 The application of various treatments resulted in a significant reduction in phytate
443 content in rice grains (Figure 7B). The results revealed that the concentration of phytic
444 acid varied from 2.33 mg kg⁻¹ (Se-NP10) to 2.9 mg kg⁻¹ in the CK, while Se-NPs10 and
445 selenite10 led to a significant reduction by 19% and 15%, respectively. These findings
446 indicate that both Se-NPs and selenite contributed in reducing the phytic acid in rice
447 grains, with the most pronounced effect observed with foliar application of Se-NPs10.
448 The reduction in phytic acid contents is a positive indicator for rice quality, as it
449 enhances the bioaccessibility of micronutrients, particularly Zn and Fe, as observed in
450 cereals (Lin et al., 2023). Previous reports have also indicated that a decrease in phytic
451 acid content could lead to the increased uptake and accumulation of Zn and Fe in wheat
452 (Lian et al., 2023).

453 The current study investigated the effects of foliar Se treatments on micronutrients,
454 revealing a significant improvement in the nutritional quality of rice through improved
455 contents of micronutrients (Zn, Fe, and Mn in rice grains (Table 2). The foliage
456 supplementation of Se-NPs10 resulted in the highest increase in Zn, Fe, and Mn
457 concentration which is consistent with the findings of Lian et al. (2023) indicating that a
458 decrease in phytic acid contents contributes to the higher levels of micronutrient in rice
459 grains. The protein content in rice grains were substantially raised with foliar
460 treatments of Se-NPs and selenite, indicating an increment of 13% with the foliar
461 application of Se-NPs 10 (Figure 7A). It is noteworthy that rice serves as the staple food
462 for over 50 % of the global community. Therefore, even a slight increase in protein

463 content or nutritional quality of rice can have a substantial impact on individuals who
464 solely rely on it (Lian et al., 2023).

465 The increased levels of micronutrients and decreased phytic acid have significant
466 implication for human's health and dietary practices. Micronutrients are essential for
467 various physiological functions in the human's body, such as metabolism, immune
468 functions and cognitive development. Foliar application of Se-NPs and selenite
469 increases the absorption of micronutrients by reducing the phytic acid content. Phytic
470 acid binds to the minerals like Zn, Fe and Mn reducing their bioavailability and
471 inhibiting their absorption in the human body (Lin et al., 2023). This can lead to
472 deficiencies of these essential micronutrients, impacting overall health and well-being.
473 The results of current study reveal that foliar supplementation of Se-NPs and selenite
474 has significant potential to impact human health by improving micronutrients intake,
475 enhancing antioxidants properties and reducing phytic acid content, all of which
476 contribute to food security and nutrition efforts.

477 **3.9 Correlations between Se and other heavy metals and nutrients concentration**

478 A direct relationship was observed between the Se concentration in polished rice
479 grains and both grain yield and elemental concentrations within the grains, as
480 evidenced by the correlation between grain Se and Zn, Fe, Mn, and protein
481 concentration (Figure 8). Conversely, a strong negative correlation was noted between
482 phytic acid contents, shoot Cd, grain Cd, Pb contents and Se concentration, consistent
483 with findings of (Hussain et al., 2020). This negative association between Se and Cd in
484 the rice grains is of significant important for food safety. Higher Se levels reduce the

485 uptake and accumulation of toxic heavy metals in rice grains. The decreased uptake of
486 heavy metals might be linked with to the higher Se levels in plant, as it down regulates
487 the Cd transporter proteins in rice, ultimately leading to decreased Cd accumulation in
488 rice grains (Zhu et al., 2022).

489 While a strong positive correlation was observed between grain Se concentration
490 and grain Zn, Fe, protein content. Se is considered essential for various ecological
491 processes, including plant development and growth, and the health of human and
492 animals. The positive correlation of Se with essential micronutrients in rice grains
493 suggests a rich ecological environment capable of supporting fauna and flora. The
494 enhancement in the essential micronutrients may be linked to higher Se levels, as Se has
495 been shown to decrease the phytic acid and improves the accumulation and
496 bioavailability of micronutrients (Lian et al., 2023). Additionally, Tang et al. (2020)
497 recently reported similar positive effects of Se on elemental concentrations. The results
498 of principal component analysis (PCA) examining the relationship between foliar
499 treatments and concentrations of other elements, as well as depiction of heavy metals,
500 revealed that PC1 and PC2 explained 58.65% and 16.02% of the variation, respectively
501 (Figure 9). This analysis confirms the positive association between Se and grain Zn and
502 Fe concentrations. Additionally, foliar application of Se led to a decrease in Cd
503 concentrations of both in grain and shoot of rice.

504 **4. Conclusions**

505 The study exhibited that foliage application of Se-NPs and selenite to late rice,
506 cultivated in moderately co-contaminated Cd and Pb soil, improved the crop yield,

507 increased the levels of essential micronutrients, facilitated Se biofortification, and
508 reduced the uptake or toxicity of heavy metals. Our findings highlight the prominent
509 impact of Se-NPs application in lowering Cd levels in rice grains, as well as Cd
510 bioavailability in Caco-2 cells. Among the treatments, Se-NPs10 exhibited lowest
511 bioaccessible and bioavailable fractions of Cd, coupled with enhanced Se bioavailable
512 concentration. Furthermore, foliar treatments exhibited significant variation in total and
513 bioavailable Cd in rice grains, emphasizing the necessity of assessing Cd
514 bioavailability through *in vivo* assays. However, additional research is necessary to
515 systematically clarify the fundamental mechanisms responsible for the decrease in Cd
516 concentration in rice grains and the effect of Se-NPs treatments on the bioavailability of
517 Cd to humans.

518 **Acknowledgements**

519 This study received support from the key project funded by Zhejiang Science and
520 Technology Bureau (2023C02002), a project granted by the Agricultural and Rural
521 Affairs Department (2020SNLF009), a project sponsored by Bijie Tobacco Company
522 (Bijie Tobacco Tech-2023-56), and the Zhejiang Province Postdoctoral Funding
523 (515000-X82301).

524 **References**

525

526 Alyemeni, M.N., Ahanger, M.A., Wijaya, L., Alam, P., Bhardwaj, R., Ahmad, P., 2018.

527 Selenium mitigates cadmium-induced oxidative stress in tomato (*Solanum*
528 *lycopersicum* L.) plants by modulating chlorophyll fluorescence, osmolyte
529 accumulation, and antioxidant system. *Protoplasma* 255, 459–469.

- 530 Arshad, A., Arshad, S., Mahmood, A.A., Asim, M.H., Ijaz, M., Irfan, H.M., Rubab, M.,
531 Ali, S., Hashmi, A.R., 2024. Zeta potential changing self-nanoemulsifying drug
532 delivery systems: A newfangled approach for enhancing oral bioavailability of
533 poorly soluble drugs. *Int. J. Pharma.* 655, 123998.
- 534 Aziz, R., Rafiq, M.T., Li, T., Liu, D., He, Z., Stoffella, P.J., Sun, K., Yang, X., 2015.
535 Uptake of cadmium by rice grown on contaminated soils and its
536 bioavailability/toxicity in human cell lines (Caco-2/HL-7702). *J. Agric. Food*
537 *Chem.* 63, 3599–3608.
- 538 Bakhtiari, M., Raeisi-Sadati, F., Raeisi-Sadati, S.Y., 2023. Foliar application of silicon,
539 selenium, and zinc nanoparticles can modulate lead and cadmium toxicity in sage
540 (*Salvia officinalis* L.) plants by optimizing growth and biochemical status. *Environ.*
541 *Sci. Pollut. Res. Int.* 30(18), 54223-54233.
- 542 Bao, S.D., 2008. *Soil Agricultural Chemistry Analysis Method*, third ed. China
543 Agriculture Press, Beijing, China (in Chinese).
- 544 Barman, F., Kundu, R., 2023. Foliar application of selenium affecting pollen viability,
545 grain chalkiness, and transporter genes in cadmium accumulating rice cultivar: A
546 pot study. *Chemosphere.* 313, 137538.
- 547 Beri, W.T., Gesessew, W.S., Tian, S., 2020. Maize cultivars relieve health risks of
548 Cd-polluted soils: in vitro Cd bioaccessibility and bioavailability. *Sci. Total*
549 *Environ.* 703, 134852.
- 550 Delaqua, D., Carnier, R., Cadore, S., Lacerda-Sanches, V., Berton, R.S., Andrade-Corbi,
551 F.C., Coscione, A.R., 2022. In vitro bioaccessibility and bioavailability of

- 552 selenium in agronomic biofortified wheat. *J. Food Comp. Ana.* 105, 104253.
- 553 Deng, X., Liu, K., Li, M., Zhang, W., Zhao, X., Zhao, Z., Liu, X., 2017. Difference of
554 selenium uptake and distribution in the plant and selenium form in the grains of
555 rice with foliar spray of selenite or selenate at different stages. *Field Crop Res.* 211,
556 165–171.
- 557 Dinh, Q.T., Cui, Z., Huang, J., Tran, T.A.T., Wang, D., Yang, W., Zhou, F., Wang, M.,
558 Yu, D., Liang, D., 2018. Selenium distribution in the Chinese environment and its
559 relationship with human health: A review. *Environ. Int.* 112, 294-309.
- 560 Fang, Y., Pan, X., Zhao, E., Shi, Y., Shen, X., Wu, J., Pei, F., Hu, Q., Qiu, W., 2019.
561 Isolation and identification of immune modulatory selenium-containing peptides
562 from selenium-enriched rice protein hydrolysates. *Food Chem.* 275, 696-702.
- 563 Farooq, M.U., Ishaq, I., Barutcular, C., Skalicky, M., Maqbool, R., Rastogi, A.,
564 Hussain, S., Allakhverdiev, S.I., Zhu, J., 2022. Mitigation effects of selenium on
565 accumulation of cadmium and morpho-physiological properties in rice varieties.
566 *Plant Phys. Biochem.* 170, 1-13.
- 567 Hamid, Y., Lin, T., Muhammad.Y., Bilal, H.,Afsheen, Z., Muhammad, Z.A., Zhen-li, H.,
568 Xiaoe, Y., 2019. Comparative efficacy of organic and inorganic amendments for
569 cadmium and lead immobilization in contaminated soil under rice wheat cropping
570 system. *Chemosphere.* 214, 259-268.
- 571 Hamid, Y., Liu, L., Haris, M., Usman, M., Lin, Q., Chen, Y.L., Rashid, M.S., Hassan,
572 Z.U., Hussain, I., Yang, X., 2023. Novel thiol-grafted composite of chitosan and
573 rice straw biochar (TH@CT-BC): A two-step fabrication for highly selective

- 574 adsorption of cadmium from contaminated water. J. Environ. Chem. Eng. 11.
575 110527.
- 576 Huang, B.Y., Zhao, F.J., Wang, P., 2022. The relative contributions of root uptake and
577 remobilization to the loading of Cd and as into rice grains: implications in
578 simultaneously controlling grain Cd and as accumulation using a segmented water
579 management strategy. Environ. Pollut. 293, 118497.
- 580 Hussain, B., Lin Q., Hamid Y., Sanaullah, M., Di, L., Hashmi, M.L.R., Khan, M.B., He,
581 Z., Yang, X., 2020. Foliage application of selenium and silicon nanoparticles
582 alleviates Cd and Pb toxicity in rice (*Oryza sativa* L.). Sci. Total Environ. 712,
583 136497.
- 584 Islam, M.Z., Park, B.J., Kang, H.M., Lee, Y.T., 2020. Influence of selenium
585 biofortification on the bioactive compounds and antioxidant activity of wheat micro
586 green extract. Food Chem. 309. 125763.
- 587 Kamran, M., Parveen, A., Ahmar, S., Malik, Z., Hussain, S., Chattha, M.S., Saleem,
588 M.H., Adil, M., Heidari, P., Chen, J.T., 2020. An overview of hazardous impacts of
589 soil salinity in crops, tolerance mechanisms, and amelioration through selenium
590 supplementation. Int. J. Mol. Sci. 21, 148.
- 591 Khanum, A., Platel, K., 2019. Bioavailability and bioactivity of selenium from wheat
592 (*Triticum aestivum*), maize (*Zea mays*), and pearl Millet (*Pennisetum glaucum*), in
593 selenium-deficient rats. J. Agric. Food Chem. 67, 6366–6376.
- 594 Kieliszek, M., Serrano-Sandoval, S.N., 2023. The importance of selenium in food
595 enrichment processes. A comprehensive review. J. Trace Ele. Med. Bio. 79,

- 596 127260.
- 597 Kong, W., Hou, X., Wei, L., Chen, W., Liu, J., Schnoor, J.L., Jiang, G., 2023.
- 598 Accumulation, translocation, and transformation of two CdSe/ZnS quantum dots
- 599 in rice and pumpkin plants. *Sci. Total Environ.* 864, 161156.
- 600 Kushwaha, A., Goswami, L., Lee, J., Sonne, C., Brown, R.J.C., Kim, K.H., 2021. Se in
- 601 soil-microbe-plant systems: sources, distribution, toxicity, tolerance, and
- 602 detoxification. *Crit. Rev. Environ. Sci. Technol.* 52, 2383-2420.
- 603 Li, D., Zhou, C., Wu, Y., An, Q., Zhang, J., Fang, Y., Li, J.Q., Pan, J., 2022. Nano
- 604 selenium integrates soil-pepper plant homeostasis by recruiting
- 605 rhizosphere-beneficial microbiomes and allocating signaling molecule levels
- 606 under Cd stress. *J. Hazard. Mat.* 432, 3894.
- 607 Li, X., Ma, L., Li, Y., Wang, L., Zhang, L., 2019. Endophyte infection enhances
- 608 accumulation of organic acids and minerals in rice under Pb^{2+} stress conditions.
- 609 *Ecotoxicol. Environ. Saf.* 174, 255–262.
- 610 Lian, J., Cheng, L., Zhai, X., Wu, R., Huang, X., Chen, D., Pan, J., Shohag, M.J.I., Xin,
- 611 X., Ren, X., He, Z., Yang, X., 2023. Zinc glycerolate (Glyzinc): A novel foliar
- 612 fertilizer for zinc biofortification and cadmium reduction in wheat (*Triticum*
- 613 *aestivum* L.). *Food Chem.* 402:134290.
- 614 Lian, J., Cheng, L., Zhai, X., Wu, R., Pan, J., Shohag, M.J.I., Xin, X., He, Z., Yang, X.,
- 615 2022. Foliar spray of combined metal-oxide nanoparticles alters the accumulation,
- 616 translocation and health risk of Cd in wheat (*Triticum aestivum* L.). *J. Hazard. Mat.*
- 617 440, 129857.

- 618 Lin, Q., Hamid, Y., Yang, H., Jiang, J., Shan, A., Wang, M., Hussain, B., Feng, Y., Li,
619 T.Q., He, Z., Yang, X., 2023. Cadmium mobility and health risk assessment in the
620 soil-rice-human system using in vitro bio-accessibility and in vivo bioavailability
621 assay: Two year field experiment. *Sci. Total Environ.* 614, 161564.
- 622 Lin, Q., Hamid, Y., Wang, H., Lu, M., Cao, X., Zou, T., Chen, Z., Hussain, B., Feng, Y.,
623 Li, T., He, Z., Yang, X., 2024. Co-foliar application of zinc and nano-silicon to rice
624 helps in reducing cadmium exposure risk: Investigations through in-vitro digestion
625 with human cell line bioavailability assay. *J. Haz. Mat.* 468, 133822.
- 626 Lin, Q., Hamid, Y., Yin, X., Hussain, B., He, Z., Yang, X., 2022. Screening of low-Cd
627 accumulating early rice cultivars coupled with phytoremediation and
628 agro-production: Bioavailability and bioaccessibility tests. *Sci. Total Environ.* 844,
629 157143.
- 630 Mao, P., Wu, J., Li, F., Li, F., Huang, R., Zhang, L., Mo, J., Li, Z., Zhuang, P., 2022.
631 Joint approaches to reduce cadmium exposure risk from rice consumption. *J.*
632 *Hazard. Mat.* 429, 128263.
- 633 Ministry of Environmental Protection and Ministry of Land and Resources of People's
634 Republic of China (2014) National survey of soil pollution. *China Environ*
635 *Protection Ind (in Chinese).* 5, 10–11.
- 636 Nedjimi, B., 2024. Selenium as a powerful trace element for mitigation of plant salt
637 stress: A review. *J. Trace Elem.Min.* 8, 100123.
- 638 Ramakrishnan, M., Arivalagan, J., Satish, L., Mohan, M., Christyraj, J., Chandran, S.A.,
639 Ju, H.J., John, L.A., Ramesh, T., Ignacimuthu, S., Kalishwaralal, K., 2022.

- 640 Selenium: a potent regulator of ferroptosis and biomass production. *Chemosphere*
641 306, 135531.
- 642 Ran, M., Wu, T.T., Jiao, Y., Wu, J., Li, J.K., 2024. Selenium bio-nanocomposite based
643 on extracellular polymeric substances (EPS): Synthesis, characterization and
644 application in alleviating cadmium toxicity in rice (*Oryza sativa* L.). *Int. J. Bio.*
645 *Macromol.* 258, 129089.
- 646 Song, J., Yu, S., Yang, R., Xiao, J., Liu, J., 2023. Opportunities for the use of selenium
647 nanoparticles in agriculture. *NanoImpact.* 31, 100478.
- 648 Stmopen, 2013. Phytic acid content in different parts of grain and its correlation with
649 rice quality traits. *Adv. J. Food Sci. Technol.* 2, 328–334.
- 650 Sun, M., Liu, G.J., Wu, Q.H., 2013. Speciation of organic and inorganic selenium in
651 selenium-enriched rice by graphite furnace atomic absorption spectrometry after
652 cloud point extraction. *Food Chem.* 141, 66–71.
- 653 Tang, L., Hamid, Y., Liu, D., Shohang, M.J.I., Zehra, A., He, Z., Feng, Y., Yang, X.,
654 2020. Foliar application of zinc and selenium alleviates cadmium and lead toxicity
655 of water spinach bioavailability/cytotoxicity study with human cell lines. *Environ.*
656 *Int.* 145, 106122.
- 657 Tokalhoğlu, Ş., Clough, R., Foulkes, M., Worsfold, P., 2014. Bioaccessibility of Cr, Cu,
658 Fe, Mg, Mn, Mo, Se and Zn from nutritional supplements by the unified BARGE
659 method. *Food Chem.* 150, 321–327.
- 660 Usman, M., Farooq, M., Wakeel, A., Nawaz, A., Cheema, S.A., Rehman, H.U., Ashraf,
661 I., Sanauallah, M., 2020. Nanotechnology in agriculture: Current status, challenges

- 662 and future opportunities. *Sci. Total Environ.* 721, 137778.
- 663 Viltres-Portales, M., Sánchez-Martín, M.J., Boada, R., Llugany, M., Valiente, M., 2024.
- 664 Liposomes as selenium nanocarriers for foliar application to wheat plants: A
- 665 biofortification strategy. *Food Chem.* 448, 139123.
- 666 Wang, C., Rong, H., Zhang, X., Shi, W., Hong, X., Liu, W., Cao, T., Yu, X., Yu, Q.,
- 667 2020. Effects and mechanisms of foliar application of silicon and selenium
- 668 composite sols on diminishing cadmium and lead translocation and affiliated
- 669 physiological and biochemical responses in hybrid rice (*Oryza sativa* L.) exposed
- 670 to cadmium and lead. *Chemosphere.* 251, 126347.
- 671 Wang, K., Fang, Q., He, P., Tu, Y., Liu, Z., Li, B., 2024. Unveiling the potential of
- 672 selenium-enriched tea: Compositional profiles, physiological activities, and health
- 673 benefits. *Trends Food Sci. Tech.* 145, 104356.
- 674 Wang, M., Mu, C., Li, Y., Wang, Y., Ma, W., Ge, C., Cheng, C., Shi, G., Li, H., Zhou, D.,
- 675 2023. Foliar application of selenium nanoparticles alleviates cadmium toxicity in
- 676 maize (*Zea mays* L.) seedlings: Evidence on antioxidant, gene expression, and
- 677 metabolomics analysis. *Sci. Total Environ.* 899, 165521.
- 678 Xiao, w., Yang, Y., Tang, N., Huang, X., Zhang, Q., Zhao, S., 2024. Innovative
- 679 accumulative risk assessment of co-exposure to Cd, As, and Pb in contaminated
- 680 rice based on their in vivo bioavailability and in vitro bioaccessibility. *Sci. Total*
- 681 *Environ.* 912, 168922.
- 682 Xiong, Y., Huang, Y., Li, L., Liu, Y., Liu, L., Wang, L., Tong, L., Wang, F., Fan, B.A.,
- 683 2023. Review of Plant Selenium-Enriched Proteins/Peptides: Extraction, Detection,

- 684 Bioavailability, and Effects of Processing. *Molecules*. 28, 1223.
- 685 Yan, G.X., Wu, L., Hou, M.y., Jia, S., Jiang, L., Zhang, D., 2024. Effects of selenium
686 application on wheat yield and grain selenium content: A global meta-analysis.
687 *Field Crops Res.* 307, 109266.
- 688 Zeng, J.Y., Guan, D.X., Dai, Z.H., Chen, J.Y., Dong, W.J., Ding, S., Ma, L.Q., 2023.
689 Selenium contents, speciation and bioaccessibility of Se-enriched rice grains from
690 Chinese markets. *Soil & Environ. Health*. 1, 100037.
- 691 Zhang, Y.M., Lin, C.Y., Li, B.Z., Cheng, Y.X., Xu, W.B., Xiao, Y., Chen, D.Y., Dong,
692 W.R., Shu, M.A., 2022. The health risk for consumers under heavy metal scenarios:
693 Reduce bioaccumulation of Cd in estuary mud crab (*Scylla paramamosain*)
694 through the antagonism of Se. *Sci. Total Environ.* 844, 157149.
- 695 Zhao, P., Huang, P., Yan, X., Chukwuma, A., Yang, S., Yang, Z., Li, H., Yang, W., 2023.
696 Inhibitory effect of exogenous mineral elements (Si, P, Zn, Ca, Mn, Se, Fe, S) on
697 rice Cd accumulation and soil Cd bioavailability in Cd-contaminated farmlands: A
698 meta-analysis. *Chemo.* 343, 140282.
- 699 Zheng, X., Zhang, Z., Chen, J., Liang, H., Chen, X., Qin, Y., Shohang, M.J.I., Wei, Y.Y.,
700 Hu, M., 2022. Comparative evaluation of in vivo relative bioavailability and in
701 vitro bioaccessibility of arsenic in leafy vegetables and its implication in human
702 exposure assessment. *J. Hazard. Mat.* 423, 126909.
- 703 Zhoua, F., Pengb, Q., Wangc, M., Liua, N., Dinhc, Q.T., Zhaia, H., Xuea, H., Liang, D.,
704 2021. Influence of processing methods and exogenous selenium species on the
705 content and in vitro bioaccessibility of selenium in *Pleurotuseryngii*. *Food Chem.*

- 706 338, 1276.
- 707 Zhu, Y., Dong, Y., Zhu, N., Jin, H., 2022. Foliar application of biosynthetic
708 nano-selenium alleviates the toxicity of Cd, Pb, and Hg in *Brassica chinensis* by
709 inhibiting heavy metal adsorption and improving antioxidant system in plant. *Eco.*
710 *Environ. Saf.* 240, 113681.
- 711 Zhuang, P., Zhang, C., Li, Y., Zou, B., Mo, H., Wu, K., Wu, J., Li, Z., 2016. Assessment
712 of influences of cooking on cadmium and arsenic bioaccessibility in rice, using an
713 in vitro physiologically-based extraction test. *Food Chem.* 213, 206–214.

Table 1. Basic physico-chemical properties of the soil used in the experiment.

Properties	Values
Soil type	Red earth
pH	5.05 ± 0.17
Organic matter (%)	6.45 ± 0.12
Total N (g kg ⁻¹)	1.47 ± 0.08
Available P (mg kg ⁻¹)	65.48 ± 2.46
Available K (mg kg ⁻¹)	159.73 ± 4.39
Total Cd (mg kg ⁻¹)	0.83 ± 0.09
Available Cd (mg kg ⁻¹)	0.38 ± 0.01
Total Pb (mg kg ⁻¹)	104.46 ± 4.03
Available Pb (mg kg ⁻¹)	24.09 ± 1.92
Total Se (mg kg ⁻¹)	0.23± 0.02

Table. 2. Macro and micro-elemental concentrations in the rice grain. Data are means \pm SD (n = 3). Different letters within same column represent the significant difference between treatments at $P < 0.05$ level

Treatments	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn(mg kg ⁻¹)	Zn (mg kg ⁻¹)	N (g kg ⁻¹)	P (g kg ⁻¹)	K (g kg ⁻¹)
CK	2.60 \pm 0.173 a	37.50 \pm 0.935 b	20.06 \pm 0.52 a	19.77 \pm 0.81 b	16.63 a	2.81 a	3.08 a
Se-NP5	2.38 \pm 0.071 ab	45.81 \pm 3.85 b	16.89 \pm 0.07 cd	22.74 \pm 1.16 b	16.04 a	2.75 a	3.28 a
Se-NP10	2.04 \pm 0.173 ab	60.51 \pm 2.21 a	18.35 \pm 0.42 bc	26.94 \pm 0.87 a	16.98 a	2.98 a	3.65 a
Selenite 5	2.15 \pm 0.214 ab	35.50 \pm 1.45 b	15.37 \pm 0.47 d	21.37 \pm 0.52 b	16.77 a	2.39 a	3.19 a
Selenite 10	1.86 \pm 0.066 ab	45.43 \pm 2.59 b	16.24 \pm 0.13 d	22.15 \pm 0.59 b	16.71 a	2.41 a	3.25 a

Figures

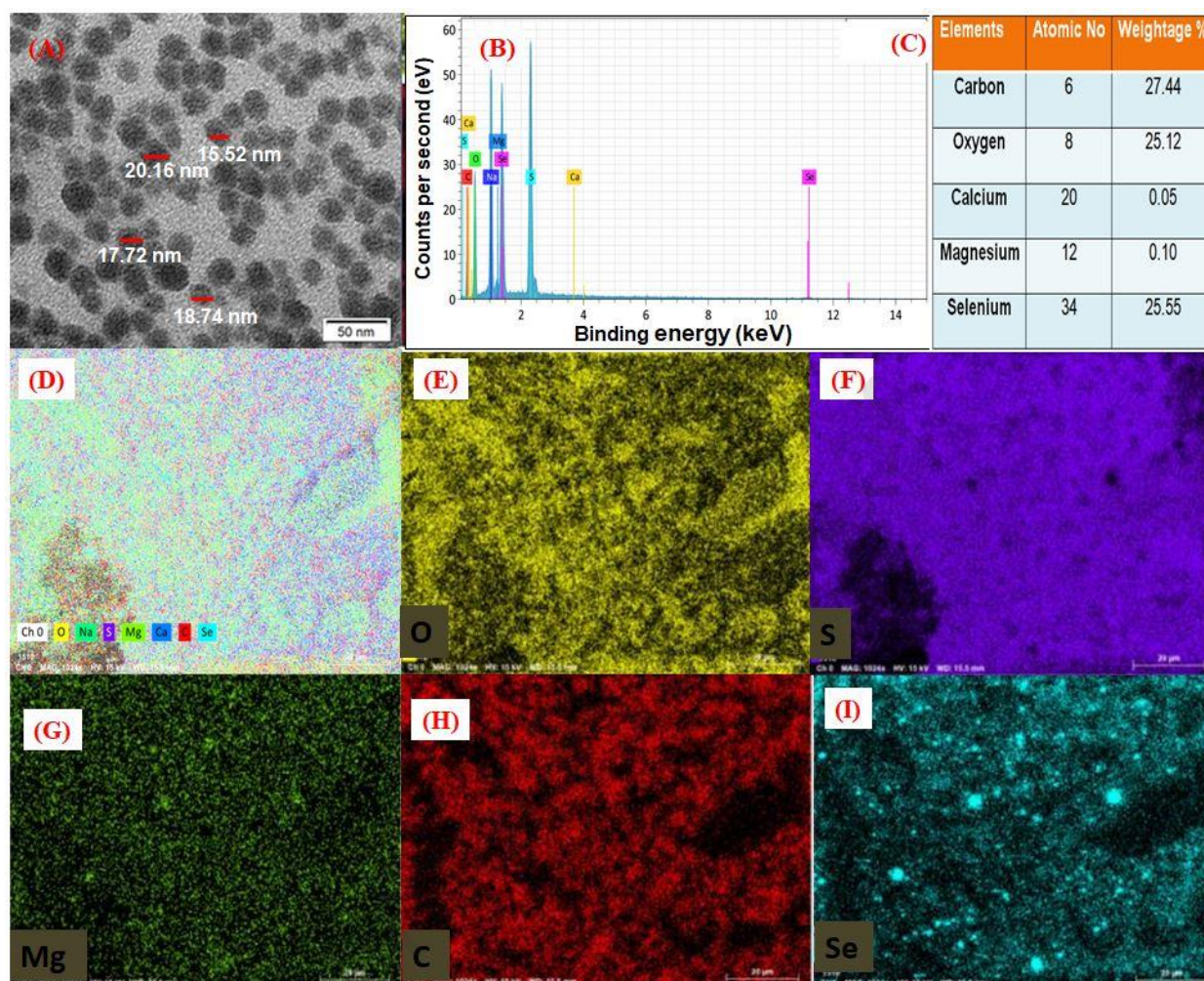


Fig.1. Transmission electron microscopy (TEM) images of selenium nanoparticles Se-NPs suspension and corresponding energy dispersive X-ray spectra (EDS) representing the percentage of element (C–I) in final suspension.

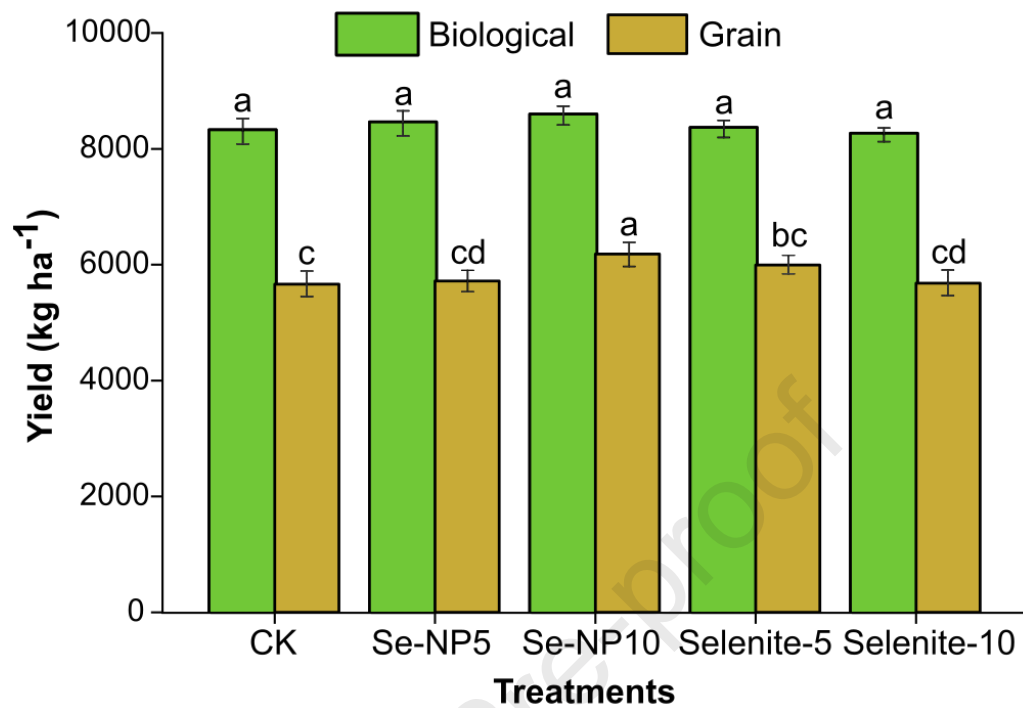


Fig. 2. Biological and grain yields of rice were assessed following foliar treatments. Presented values are means of three replicates while error bar represents the standard error of the replicates. Different letters represent the significant difference between treatments at $P < 0.05$ level. Here Se-NPs5: Selenite 5 = 5 mg L⁻¹ and Se-NPs 10: Selenite 10 = 10 mg L⁻¹

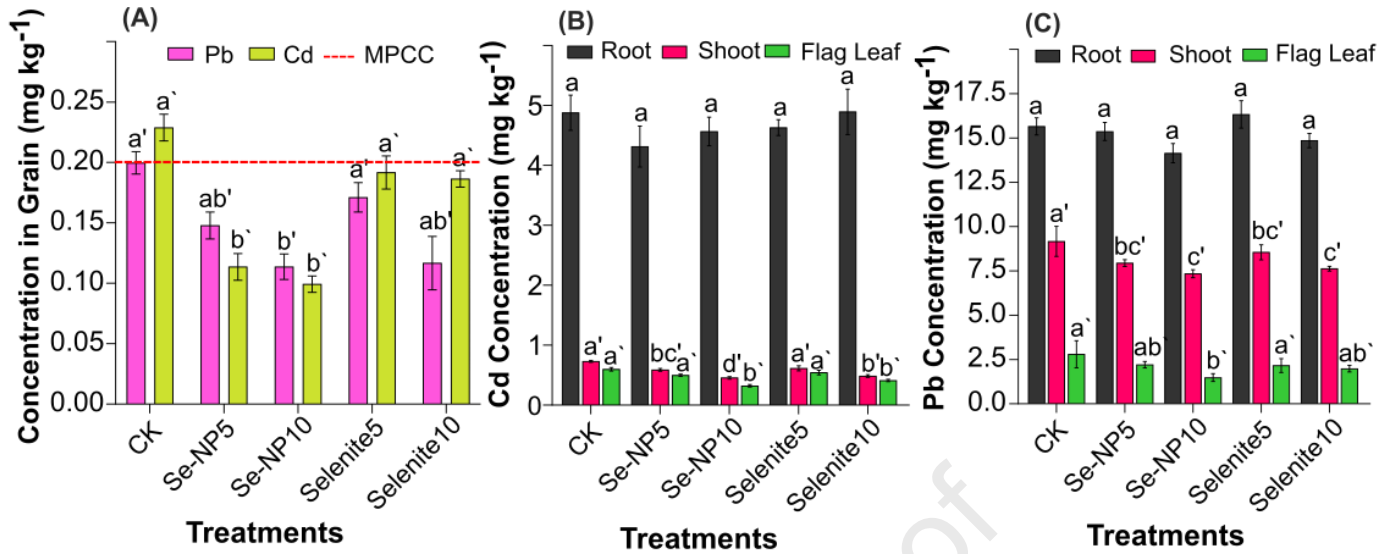


Fig.3. Cd and Pb concentrations in the grain (A) root, shoot and flag leaf (B, C) for rice following foliar treatments. Presented values are means of three replicates while error bar represents the standard error of the replicates. Different letters represent the significant difference between treatments at $P < 0.05$ level. Here Se-NPs5: Selenite 5 =5mg L⁻¹ and Se-NPs 10: Selenite 10 =10 mg L⁻¹. Maximum permissible concentration in China (MPCC)

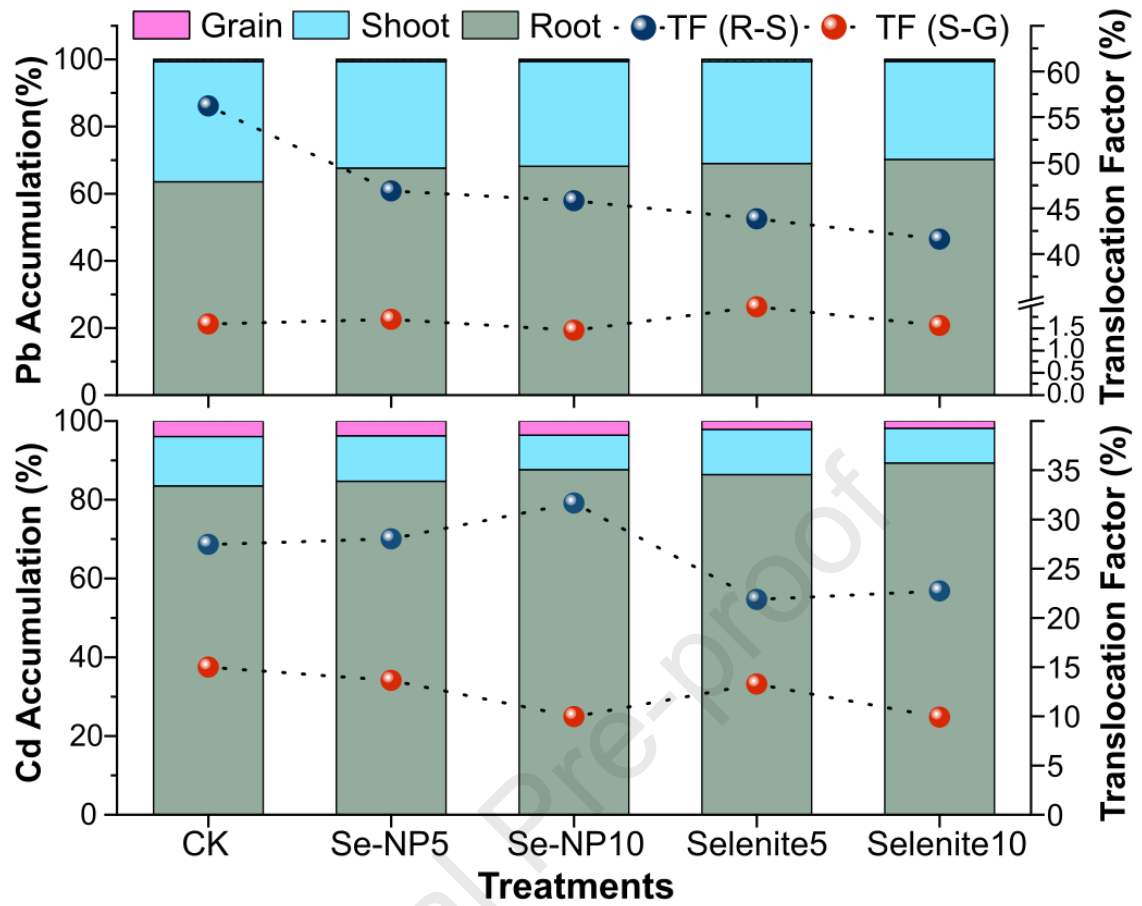


Fig.4. Total Cd and Pb accumulation in the root, shoot, and grains of rice and translocation factor (%) from root-shoot and shoot to grains following foliar treatments. Presented values are means of three replicates. Here Se-NPs5: Selenite 5 = 5mg L⁻¹ and Se-NPs 10: Selenite 10 = 10 mg L⁻¹.

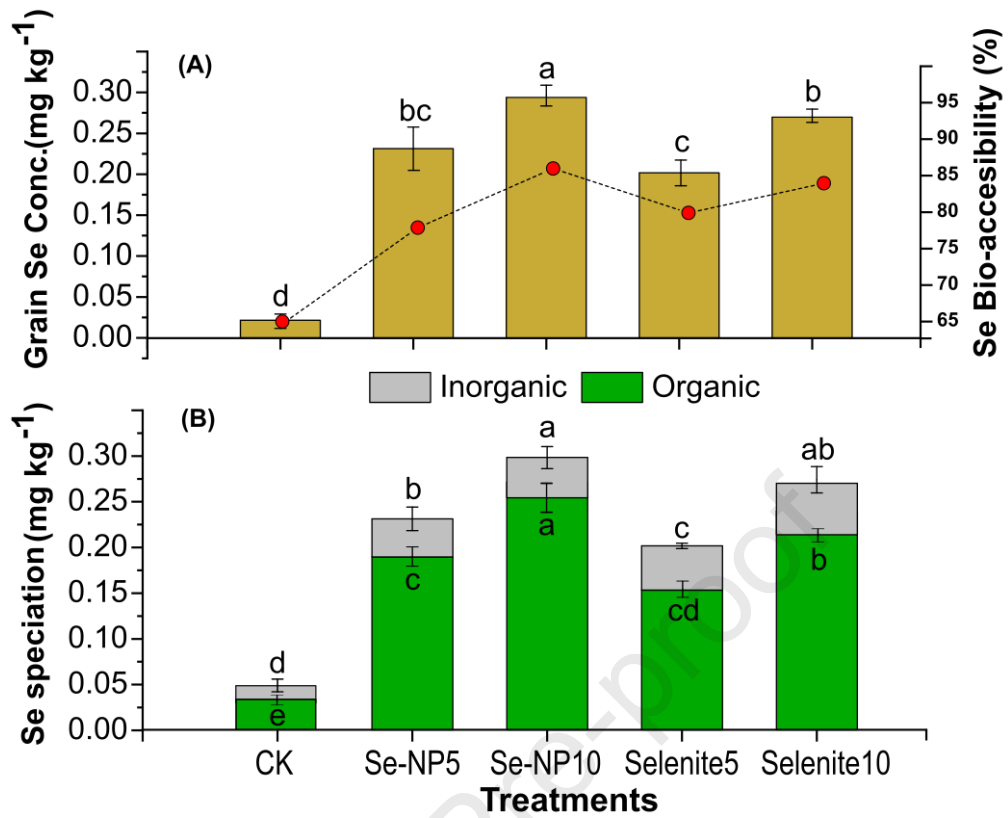


Fig.5. Selenium concentration and speciation in rice grains following foliar treatments. Presented values are means of three replicates while error bar represents the standard error of the replicates. Different letters represent the significant difference between treatments at $P < 0.05$. Here Se-NPs5: Selenite 5 = 5 mg L^{-1} and Se-NPs 10: Selenite 10 = 10 mg L^{-1} .

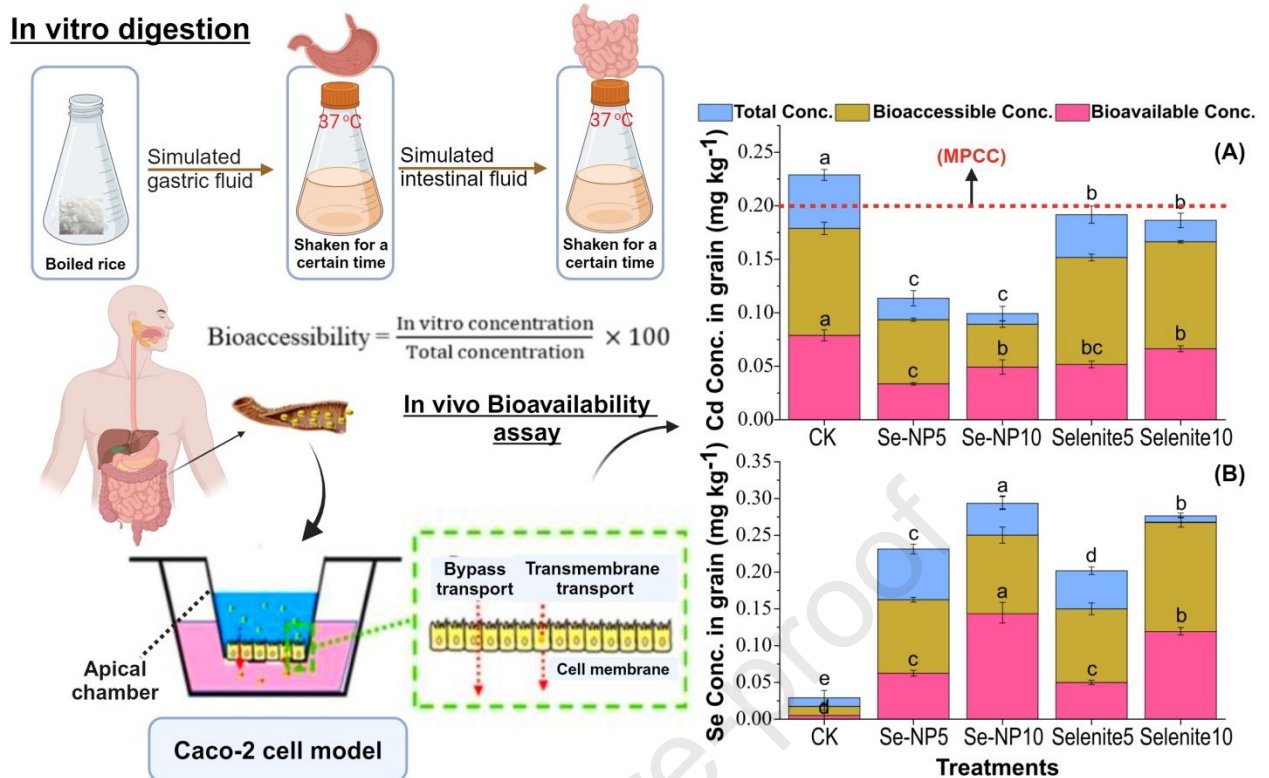


Fig.6. Bioaccessible and bioavailable Cd and Se concentrations (A, B) respectively in rice grains following foliar treatments. Presented values are means of three replicates while error bar represents the standard error of the replicates. Different letters represent the significant difference between treatments at $P < 0.05$. Here as Se-NP5: Selenite 5 = 5 mg L^{-1} and Se-NP10: Selenite 10 = 10 mg L^{-1} .

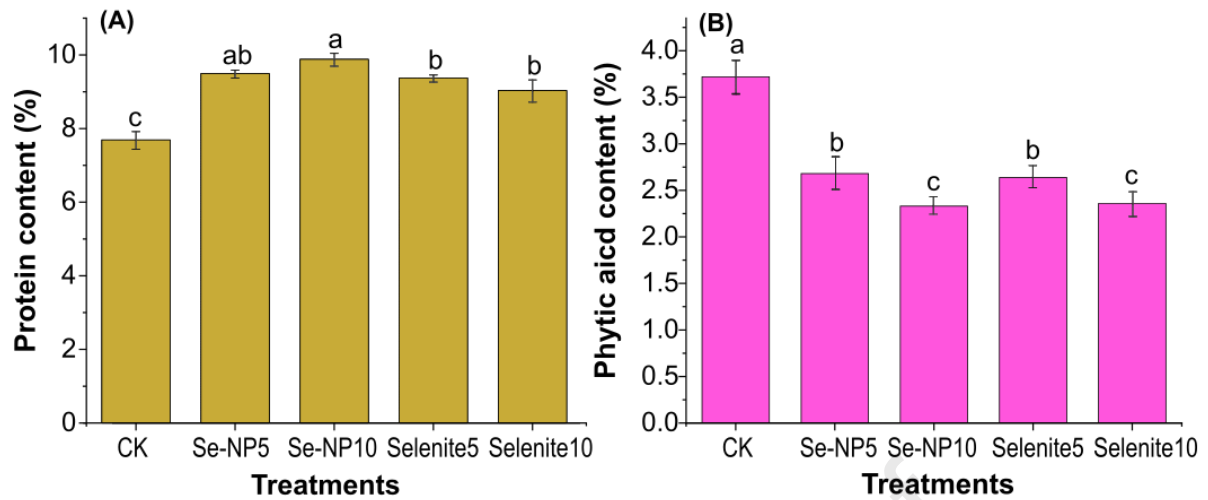


Fig.7. Rice nutritional quality protein content (A) and phytic acid (B) respectively in rice grains following foliar treatments. Presented values are means of three replicates while error bar represents the standard error of the replicates. Different letters represent the significant difference between treatments at $P < 0.05$. Here as Se-NPs5: Selenite 5 = 5mg L^{-1} and Se-NPs 10: Selenite 10 = 10 mg L^{-1} .

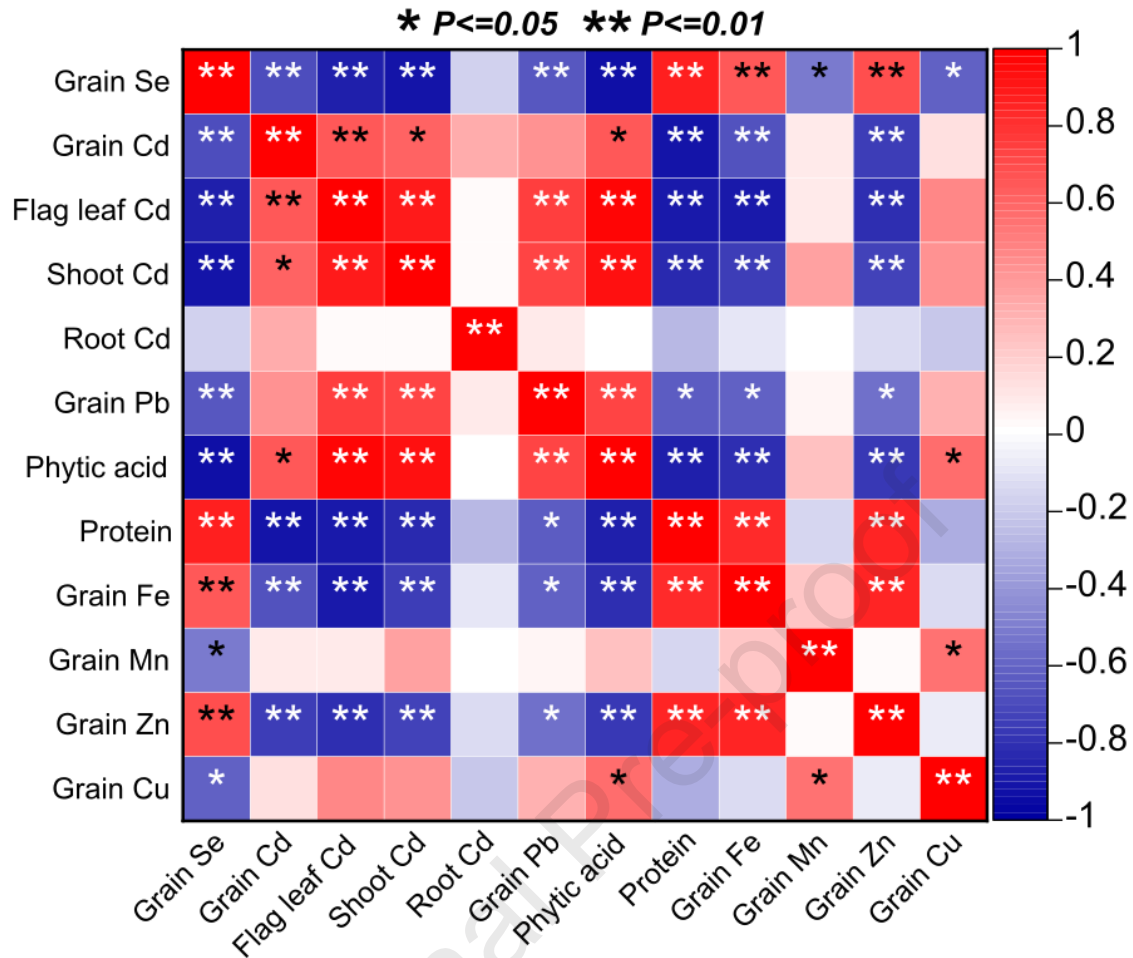


Fig.8. Pearson correlation between rice grains Se concentration and root, shoot and grains Cd, grains Pb, phytic acid and protein content, micronutrients Zn, Fe and Mn.

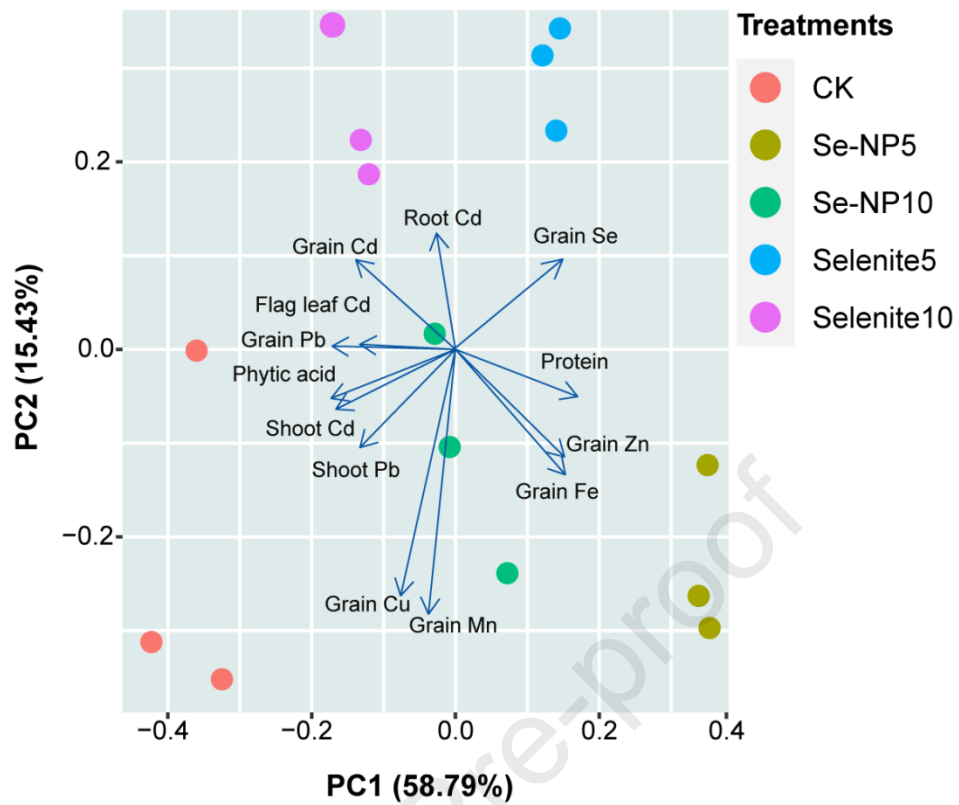


Fig.9. Principal component analysis of 15 samples corresponding to 5 treatments to the first (PC1) and second (PC2) principal components.

Highlights

- Foliar application of Se-NPs and selenite effectively achieved the safe rice production
- Se-NPs and selenite have shown potential in reducing the bioavailability of Cd in Caco-2 cells
- Se-NPs and selenite improved the rice quality and promoted the Se biofortification
- Se-NPs restricted the heavy metals translocation between the plant parts

Declaration of interest's

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The authors declare they have no known competing financial interests or personal relationships that could influence the work reported in this paper

Journal Pre-proof