



Research article

Effects and mechanisms of different exogenous organic matters on selenium and cadmium uptake by rice in natural selenium-cadmium-rich soil

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ARTICLE INFO

Keywords:

Selenium

Cadmium

Rice

Exogenous organic matters

Non-rhizosphere

Rhizosphere

ABSTRACT

Many natural selenium (Se)-rich rice plants are being polluted by cadmium (Cd). In this study, for reducing Cd concentrations in rice grains while maintaining Se concentrations, the effects of different exogenous organic matters (OMs), such as humic acid (HA), cow manure (CM), and vermicompost (VC), on Se and Cd uptake in rice growing in natural Se-Cd-rich paddy soils were investigated by pot experiments. The Se and Cd concentrations in the soil solution, their species in the soil, and their concentrations and translocations in rice tissues were determined. Results showed that different exogenous OMs exhibited distinct percentage changes in Se and Cd levels in rice grains with amplitudes of -19.42% and -56.90% (significant, $p < 0.05$) in the HA treatments, $+10.79\%$ and -1.72% in the CM treatments, and $+15.83\%$ and -15.52% in the VC treatments, respectively. Correlation analysis showed that the concentrations of Se and Cd in rice grains might be primarily influenced by their concentrations in the soil solution, rather than the Se/Cd molar ratios in the soil solution or their translocations in rice tissues. HA decreased Se and Cd bioavailability in soil by increasing HA-bound Se and residual Cd, respectively. Meanwhile, HA increased soil solution pH, which was negative for Cd bioavailability but positive for Se bioavailability. This additive effect made HA lowered Cd concentration more than Se concentration in both soil solution and grain. CM and VC did not have this additive effect and thus have limited effects on grain Se and Cd concentrations. In addition, according to grain Se and Cd concentrations, to prioritize reducing Cd in rice, use HA; to prioritize increasing Se in rice, use VC. This study enhances the understanding of Se and Cd uptake mechanisms in rice with the applications of various OMs and offers potential remediation methods for Se-Cd-rich paddy soils.

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<https://doi.org/10.1016/j.heliyon.2024.e37740>

Received 22 August 2024; Received in revised form 1 September 2024; Accepted 9 September 2024

Available online 10 September 2024

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1. Introduction

Selenium (Se) is an essential trace element for humans and has crucial biological functions, such as antioxidant, anticancer, and improving human immunity [1–3]. Se deficiency in humans can lead to Keshan disease and Kashin-Beck disease [4]. Currently, approximately 1 billion people in the world experience Se deficiency caused by insufficient uptake of this essential element [5]. In China, the per capita Se intake is only about half of the world average, and about 500–600 million people are Se deficient [5]. Therefore, enhancing Se intake in China people holds significant implications for improving global per capita Se intake and helping to reduce the number of people with Se deficiency worldwide.

Rice is a staple food in China [6], and consuming Se-rich rice has the potential to improve the per capita Se intake and reduces the risk of Se deficiency in the human body [7,8]. However, recent research has indicated that Se-rich rice often has higher concentrations of cadmium (Cd) compared to Se-normal rice [9–12]. A previous study showed that Cd concentrations were about 4.55 times higher in Se-rich rice grains (314 samples) than in common rice grains (294 samples) in China [10], which is of greater concern in various representative Se-enriched regions in China, where Cd contamination was detected in 24%–56% of Se-rich rice samples [13–15]. Cd, as a class 1 carcinogen (human carcinogen), is widely recognized as the most hazardous and mobile element [16,17], which is easily enriched in rice grains [18–20]. Se is easily enriched in mineral deposits and stone coals which are also easily enriched with Cd [21]. These types of deposits and stone coal contribute to high levels of Se and heavy metals in their weathering products (such as soil), which lead to the enrichment of heavy metals (such as Cd) in natural Se-enriched soils and make the local rice have high levels of Se and Cd [9,22,23]. Therefore, it is important to conduct studies in Se-Cd-rich paddy fields to prevent Cd accumulation in rice while still allowing for Se uptake.

Organic matter (OM) is the main constituent of soil, and using OM as a fertilizer has the potential to enhance soil physical and chemical properties, thereby improving crop yield and quality [24]. These benefits make OM indispensable in agricultural production [25]. OM can also influence the bio-availabilities of the elements in the soil [24]. For example, in waterlogged environments, the presence of OM can influence the bio-availability of Se and Cd in soil by complexation, dissolving iron (hydro)oxides, and changing soil pH. By complexation, high molecular weight OMs decreased the bioavailability of Se and Cd in soil, but low molecular weight OMs did the opposite [26,27]. OM promotes the reduction and dissolution of iron (Fe) (hydro)oxides, thereby accelerating the release of Se and Cd from Fe (hydro)oxides into soil solution [21]. Additionally, the application of OM increased soil pH by promoting the microbe-mediated reduction reactions under anaerobic waterlogged conditions, and accordingly increased (or decreased) Se (or Cd) concentrations in soil solution [27–29]. Meanwhile, in soil solution, the changes in Se and Cd concentrations significantly influence Se/Cd molar ratios and then affect the uptake of these two elements by rice [9,30]. However, previous studies primarily focused on single high-Se [31–34] or single high-Cd [27,35–37] conditions, and these results can not be used to speculate the underlying impact of OM on Se and Cd uptake by rice growing in naturally Se-Cd-rich soils. Therefore, the impact of OM on Se and Cd uptake by rice grown in paddy land containing high levels of these two elements from natural origins remains unclear.

In addition, OMs from different sources typically exhibit variations in composition, primarily manifested by differences in molecular weights [24,28]. Humic acid (HA) often possesses a high molecular weight, leading to its propensity for precipitation within the soil, decreasing the solubility of Se and Cd through complexation using functional groups, and subsequently reducing their bio-availabilities [35,38,39]. Conversely, other types of OMs (such as manure) not only contain high molecular weight OMs (such as HA) but also encompass a rich abundance of soluble low molecular OMs [26,28]. The bio-availabilities of Se and Cd in soil are often enhanced by these low molecular weight OMs, which frequently contend with these two elements for adsorption sites [26,27]. Hence, it is imperative to investigate the impacts of OMs derived from various origins on the absorption of Se and Cd by rice, and the results are crucial to identify the most appropriate OM for the remediation of paddy soils abundant in both Se and Cd.

In this study, the effects of exogenous OMs, such as HA, cow manure (CM), and vermicompost (VC), on the yield, as well as the uptake of Se and Cd in rice grown in paddy soils that are naturally high in Se and Cd, were studied through pot experiments. If some OMs inhibited Cd uptake by rice without affecting Se uptake, then the utilization of these OMs could have the potential to be employed to address the issue of Cd pollution in Se-rich paddy fields. Conversely, if Se and Cd uptake by rice is promoted, special attention should be paid when using this kind of OM. These findings have both theoretical and practical significance in developing remediation plans for natural Se-Cd-rich paddy fields.

2. Materials and methods

2.1. Experimental materials

We collected paddy soils by sampling the uppermost layer at a depth of 0–20 cm in Shuang'an Town, Ziyang County, Ankang City, Shaanxi Province, China. Shuang'an Town is a representative region characterized by elevated Se levels with certain areas being affected by Cd contamination. The soils that were gathered were dried by exposure to air and then passed through a 2 mm nylon sieve. The different types of OM (HA, CM, and VC) used in this study were purchased from MACKLIN Ltd., Shandong Lvditou Ltd., and Shanghai Wolv Ltd., respectively. A rice variety called Taiyou 068, obtained from a seed company in Ankang City, was utilized. Se concentrations in soil, HA, CM, and VC were 3.75 mg kg⁻¹, 0.24 mg kg⁻¹, 1.20 mg kg⁻¹, and 0.55 mg kg⁻¹, respectively. Cd concentrations in soil, HA, CM, and VC were 3.31 (mg kg⁻¹), 0.49 (mg kg⁻¹), 0.33 (mg kg⁻¹), and 0.23 (mg kg⁻¹), respectively. OM concentrations in soil were 12 g kg⁻¹. The major element concentrations of each OM are shown in Table S1. The spectra of Fourier transform infrared (FTIR) spectrometer of OMs used in this study were shown in Fig. S1-Fig. S3, and the functional groups were

identified according to the results of previous studies [40,41].

2.2. Rice pot experiments

Briefly, our study incorporated four treatments: control (CK) treatment (adding no exogenous OM), HA treatment (adding HA), CM treatment (adding CM), and VC treatment (adding VC). The quantity of OM added was equivalent to 1 % of the soil's total mass. Each treatment was replicated three times. The details are given below.

The pot trials took place in a natural climate greenhouse situated near the Ankang Institute, which is part of China University of Geosciences, located in Ankang City, Shaanxi Province, China. The rice seeds underwent surface sterilization through immersion in a 10 % (v/v) H_2O_2 solution for a duration of 15 min. Following the process of sprouting, the young plants were carefully raised in a protected location for approximately 30 days [42].

In this research, a culture system called compartmentalized rhizo-bag was utilized (Fig. 1). Each pot had dimensions of 25 cm (height) and 20 cm (diameter), containing a total of 6 kg of soil. A portion (1.5 kg) of total soil was subsequently transferred into a nylon mesh bag measuring 15 cm in height and 10 cm in diameter. Then, the bags were positioned at the pot's center to create a rhizosphere environment [36,43]. Water and dissolved nutrients could pass through the mesh due to its pore size of 48 μm , while root penetration was prevented. To treat the soil batches, HA, CM, and VC were thoroughly blended into the soils at a concentration of 1 % (w/w). There was also a treatment conducted under identical termination conditions, where no exogenous OM was added, and this treatment was defined as the CK treatment. Overall, we prepared four treatments, which were CK treatments, HA treatments, CM treatments, and VC treatments. A set of 12 pots was acquired by duplicating every treatment thrice. In each pot, the doses of 0.20 g N kg^{-1} as $\text{CO}(\text{NH}_2)_2$, 0.12 g P kg^{-1} as KH_2PO_4 , and 0.26 g K kg^{-1} as KCl were evenly applied as the fundamental fertilizers [44]. Then, we initially flooded the soil with ultrapure water until it surpassed the soil layer by approximately 3 cm in depth. This allowed for an equilibration period lasting three days before proceeding with transplanting rice seedlings into each rhizo-bag (one plant per pot). During the entire growth phase of the rice plants, flooding conditions were sustained by maintaining the ultrapure water level slightly higher than the soil layer, at approximately 3 cm. In order to gather soil solutions from areas far and near the roots, we employed soil pore-water sampling devices (Wageningen, the Netherlands) that were buried in both non-rhizosphere and rhizosphere areas.

After being transplanted in the pots, rice samples (including roots, straw, husks, and grains) were collected 120 days later. Subsequently, they were washed extensively using both tap water and ultrapure water. Next, the botanical samples were dried in an oven at 75 °C until they attained a consistent mass. Finally, desiccated samples were finely pulverized into a powdery state. Meanwhile, soil samples from both the non-rhizosphere and rhizosphere areas were carefully taken from each pot at day 120. After drying in the air, the soil samples were pulverized into a fine powder. Soil solution samples were also collected by soil pore-water sampling devices at various time points after transplanting, specifically on day 10, day 20, day 40, day 80, and day 120.

2.3. Fractionation of Se and Cd in soil

In order to obtain the Se species from soil samples, the sequential extraction method described in previous studies was employed with minor modification [21,45,46], including water-soluble Se, ligand-exchangeable Se, fulvic acid (FA)-bound Se, HA-bound Se, iron (Fe)-manganese (Mn)-oxide-bound Se, and residual Se. Briefly, Water-soluble Se was obtained from the soil by mixing it with ultrapure water (solid-to-liquid ratio 1:10). Ligand-exchangeable Se was extracted using a buffer solution consisting of 0.1 M $\text{KH}_2\text{PO}_4 + \text{K}_2\text{HPO}_4$ at pH 7 (solid-to-liquid ratio 1:10). OM-bound Se was extracted using a solution of 0.1 M NaOH (solid-to-liquid ratio 1:10), followed by heating in a water bath at a temperature of 90 °C for a duration of 2 h. The OM-bound Se was further divided into two parts: one

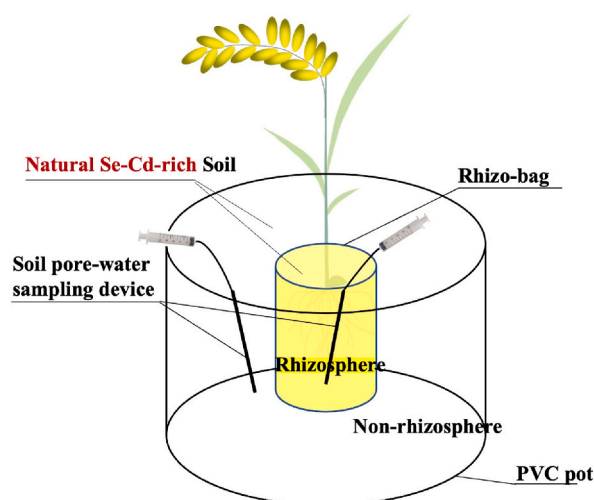


Fig. 1. Schematic diagram of the planting method.

containing fulvic acid (FA)-bound Se and the other containing HA-bound Se. To separate the two fractions, the pH of the solution was modified to acidic conditions (pH 1) using 6 M HCl, resulting in precipitation of dissolved HA. After standing overnight, the supernatant representing FA-bound Se was obtained by subjecting the mixture to centrifugation. The precipitate containing the HA-bound Se fraction was retained. Fe-Mn-oxide-bound Se was extracted by 20 mL of 3 mol L⁻¹ HCl. Finally, residual Se in the soil was treated by digestion with a mixture of HNO₃, HF, and HClO₄.

The extraction of Cd species in soil was conducted following the protocols outlined by previous studies [47,48]. Briefly, 1.00 g of soil was subjected to successive extractions using 20 mL of 1 M MgCl₂ to extract exchangeable Cd. Subsequently, carbonate Cd was extracted by treating the soil with 20 mL of 1 M CH₃COONa (pH 5). Reducible Cd was then extracted by employing a solution consisting of 0.04 M NH₂OH·HCl in 25 % (V: V) HOAc. Oxidizable Cd was extracted by 3 mL of 0.02 M HNO₃ and 5 mL of 30 % H₂O₂, and subsequent extraction with 15 mL of 1 M NH₄OAc. Finally, residual Cd was extracted using HClO₄-HNO₃-HCl digestion solution.

2.4. Chemical analysis

Soil solution pH was determined quickly after collection, and the pH of the soil was measured at a soil/water ratio of 1:2.5 using a pH meter (INESA). Analytical-grade reagents were used for all experiments, and all solutions were produced by Milli-Q water (18.25 MΩ cm). FTIR (ThermoFisher, Nicolet IS50) was employed to characterize the functional groups of HA, CM, and VC.

Rice samples (roots, straw, husks, and grains; 0.1 g) were weighed and digested in 25 mL containers made of Teflon material that could be securely closed. A combination of HNO₃ (4 mL) and HClO₄ (1 mL) was added to the containers along with the samples. Then, the containers were heated at 160 °C in an oven for 12 h [38]. Soil samples collected from non-rhizosphere and rhizosphere areas were also digested in 25 mL Teflon containers, similar to the method used for rice samples. However, there were variations in the composition of the digestion solutions (2 mL HNO₃, 1 mL HF, and 1 mL HClO₄), as well as differences in the digestion temperature set at 180 °C and the duration of 48 h. Soil solution samples collected from non-rhizosphere and rhizosphere (1–2 mL) areas were evaporated to near dryness at 100 °C. Subsequently, they were digested using 2 mL HNO₃ plus 1 mL H₂O₂ at room temperature for 2 h and then at 100 °C on an electric hotplate.

Se in digestion solutions was reduced from Se⁶⁺ to Se⁴⁺ by adding 1 mL 6 M HCl at 90 °C for 30 min, and then diluted to achieve the desired volume, containing 5 % HCl, and finally determined using hydride-generation atomic fluorescence spectrometry (HG-AFS; Haiguang HGF-V). Fe and Cd in digestion solutions were diluted to achieve the desired volume with 2 % HNO₃, and were then determined using inductively coupled plasma optical emission spectrometry (ICP-OES; iCAP PRO XP) and inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7800), respectively.

The retrieval percentages of elements in the standard reference materials (GBW07904 for soil samples, and GBW10230 and GBW10022 for plant samples) were found to be within the error range. The recovery rates for Se ranged from 93.16 % to 110.26 %, while for Cd they ranged from 88.20 % to 110.83 %.

2.5. Data analysis

Element concentrations in rice tissues and rice yields were both based on dry weight. To compare the average values among treatments, we conducted a one-way ANOVA with multiple comparisons using Tukey's HSD test. Spearman's correlation coefficient was employed to evaluate the association between the two indicators (pH, Se concentrations, Cd concentrations, and Fe concentrations).

The translocation factors (TFs) were employed for the assessment of Se and Cd translocation ability between different adjacent rice tissues. The estimation of TFs for Se or Cd was conducted utilizing the following equation:

$$TF_{a-b} = C_b/C_a$$

where C denotes Se/Cd concentration, and a and b represent different adjacent rice tissues.

3. Results

3.1. FTIR spectra of HA, CM, and VC

FTIR spectra showed that HA has O-H stretching of various hydroxyl functional groups (3421.68 cm⁻¹), C-H stretching in methyl groups of aliphatic molecules (2928.57 cm⁻¹), C=C and COO⁻ stretching in aromatic rings (1578.87 cm⁻¹), C-H deformation vibration CH₂ units and COO⁻ stretching vibration (1384.60 cm⁻¹), and C-O stretching vibration (1039.13 cm⁻¹) (Fig. S1); CM has O-H stretching of various hydroxyl functional groups (3417.72 cm⁻¹), C-H stretching in methyl groups of aliphatic molecules (2926.22 cm⁻¹), C=O stretching vibration (1648.27 cm⁻¹), C=C and COO⁻ stretching in aromatic rings (1510.69 cm⁻¹), C-H and OH stretching vibration (1421.10 cm⁻¹), and C-O stretching vibration (1088.84 cm⁻¹) (Fig. S2); VC has O-H stretching of various hydroxyl functional groups (3422.30 cm⁻¹), C-H stretching in methyl groups of aliphatic molecules (2919.54 cm⁻¹), C=O stretching vibration (1639.70 cm⁻¹), C-H and OH stretching vibration (1434.80 cm⁻¹), C-H deformation vibration CH₂ units and COO⁻ stretching vibration (1384.86 cm⁻¹), and C-O stretching vibration (1031.82 cm⁻¹) (Fig. S3) [40,41].

3.2. Rice yield

In comparison to the CK treatments, the applications of exogenous OMs (such as HA, CM, and VC) led to different levels of slight enhancement in the rice biomass but without statistically significant difference ($p > 0.05$) (Fig. 2; Table S2). Specifically, the grain yields (g plant^{-1}) were 26.90, 30.28, 28.13, and 30.97 in the CK, HA, CM, and VC treatments, respectively (Fig. 2 d).

3.3. pH and the concentrations of Se, Cd, and Fe in soil solution

The results showed that the soil solution's pH experienced an upward trend across all treatments during the growth of the rice plant (Fig. 3a and b; Table S3; Table S4), with slightly higher pH levels in the rhizosphere (7.49–8.09) than in the non-rhizosphere samples (7.49–7.79) (Fig. 3a and b). Among the treatments, the soil solution pH was highest in the HA treatments, followed by the CM treatments, VC treatments, and CK treatments (Fig. 3a and b; Table S3; Table S4).

In the soil solution, there was an initial rise in Se levels observed during the first 20 days, which was then followed by a gradual decrease towards a relatively stable level (Fig. 3c and d; Table S3; Table S4). In the non-rhizosphere, both CM and VC treatments had higher soil solution Se concentrations compared to HA and CK treatments, from day 0 to day 20 (Fig. 3 c). In the soil solution of the rhizosphere, VC treatments exhibited the highest levels of Se concentrations, followed by CK, CM, and HA treatments within the first 20 days (Fig. 3 d). From day 40 to day 120, no significant differences existed in soil solution Se concentrations among all treatments ($1.78\text{--}1.86 \mu\text{g L}^{-1}$ in non-rhizosphere and $1.85\text{--}1.99 \mu\text{g L}^{-1}$ in rhizosphere samples) (Fig. 3c and d).

The Cd concentrations in the soil solution initially increased and then decreased in the CK, HA, and VC treatments. However, Cd concentrations increased overall in the CM treatments, along with the growth period (Fig. 3e and f; Table S3; Table S4). The trends of soil solution Cd concentrations were complex among these treatments, but generally similar between the non-rhizosphere and rhizosphere samples (Fig. 3e and f). Throughout most stages of the growth period, the CK treatments had the highest soil solution Cd concentrations, followed by the HA and VC treatments. At the beginning of transplanting, soil solution Cd concentrations in the CM treatments were similar to those observed in the other treatments. However, over time, they gradually increased and eventually reached the highest concentration (Fig. 3e and f).

Regarding the Fe concentrations in the soil solution, we found that they increased initially (day 0-day 20) and then decreased (day 40-day 120) but were higher in non-rhizosphere than in rhizosphere samples to some extent (Fig. 3g and h; Table S3; Table S4). In most stages of the growth period, VC and CM treatments had the highest soil solution Fe concentrations, followed by CK and HA treatments (Fig. 3g and h).

Finally, we conducted an examination of the correlation between Se, Cd, and Fe concentrations, as well as pH levels in the soil solutions from both non-rhizosphere and rhizosphere samples collected simultaneously (Table S5; Table S6). The results demonstrated that significant correlations ($p < 0.05$) only existed in the non-rhizosphere soil solution at day 10 (Se with Fe, $r = 0.671$), day 20 (Se with Fe, $r = 0.783$), and day 80 (Cd with Fe, $r = 0.732$) (Fig. 4; Table S5; Table S6).

3.4. Se/Cd molar ratios in soil solutions

The values of Se/Cd molar ratios in soil solutions were initially elevated and then decreased (Fig. 5; Table S7). Among the non-rhizosphere soil solutions, VC treatments had the highest Se/Cd molar ratios, followed by HA and CK treatments, except at day 10 (Fig. 5 a). The soil solutions of CM treatments initially exhibited higher Se/Cd molar ratios compared to those of the CK treatments but

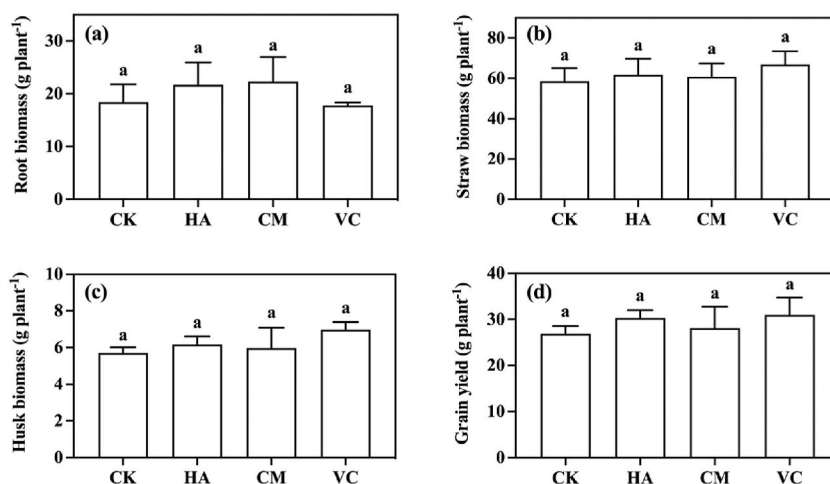


Fig. 2. Effects of exogenous OMs on the biomass of root (a), straw (b), husk (c), and grain (d) (g plant^{-1}). Values are recorded as mean \pm SD, $n = 3$. Distinguishing lowercase letters denote notable distinctions between treatments with a significance level of $p < 0.05$ or $p < 0.01$ (one-way ANOVA with Tukey's HSD).

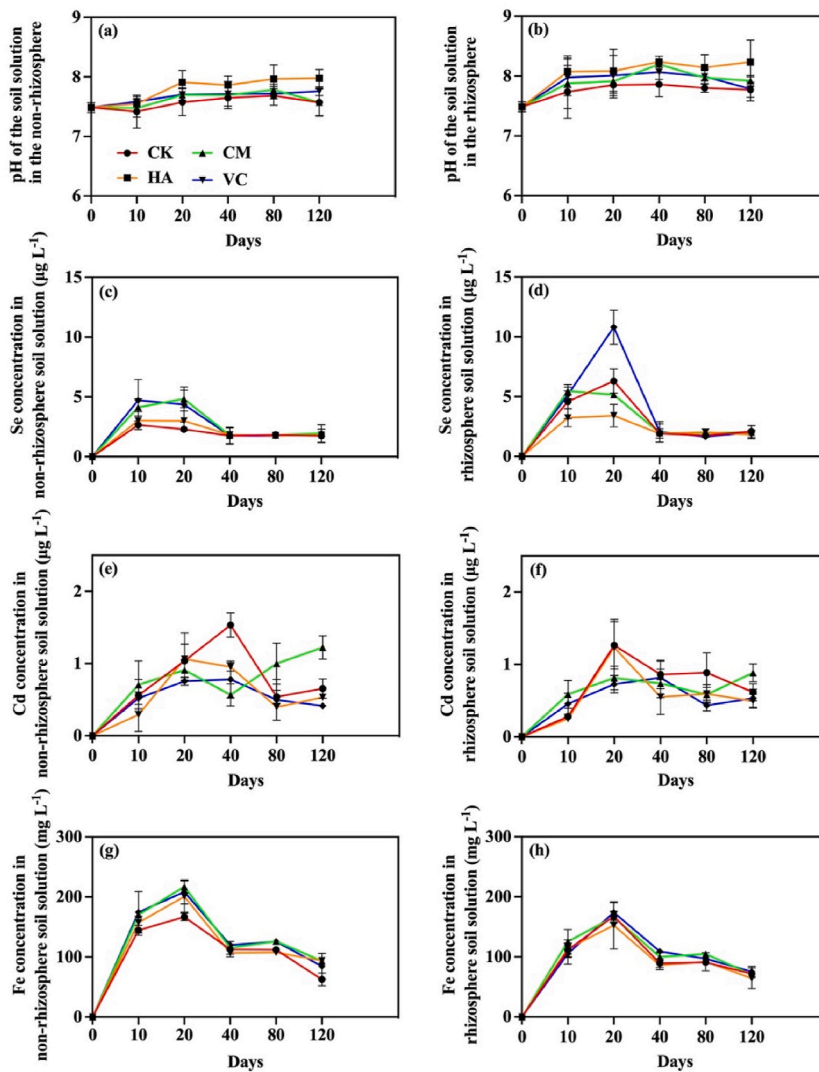


Fig. 3. Dynamics of pH, selenium (Se), cadmium (Cd), and iron (Fe) in soil solution in non-rhizosphere (a, c, e, g) and rhizosphere (b, d, f, h) samples due to the application of different types of exogenous organic matters (OMs). Values are recorded as mean \pm SD, n = 3. At day 0, an element concentration of 0 represents unsampled.

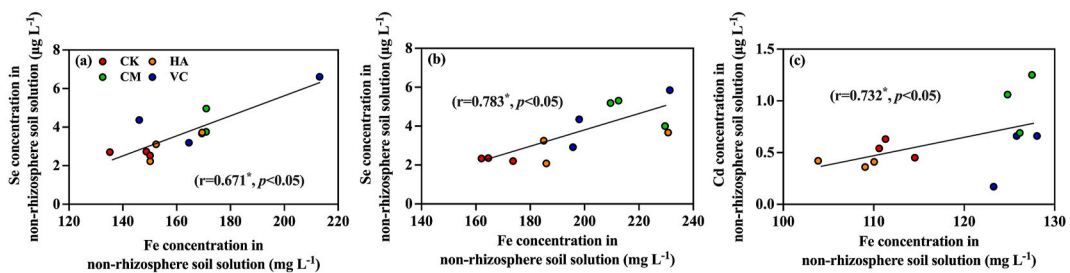


Fig. 4. Scatter diagrams. The correlations of concentrations between Fe and other elements (Se and Cd) in non-rhizosphere soil solution at day 0 (a), day 20 (b), and day 80 (c). Significance is represented as * (or **) at the $p < 0.05$ (or $p < 0.01$) level.

gradually became the lowest (Fig. 5 a). In the rhizosphere soil solutions, VC treatments had higher Se/Cd molar ratios than those of CM and HA treatments (Fig. 5 b). CK treatments had the highest Se/Cd molar ratios at day 10, which then decreased with rice growth (Fig. 5 b).

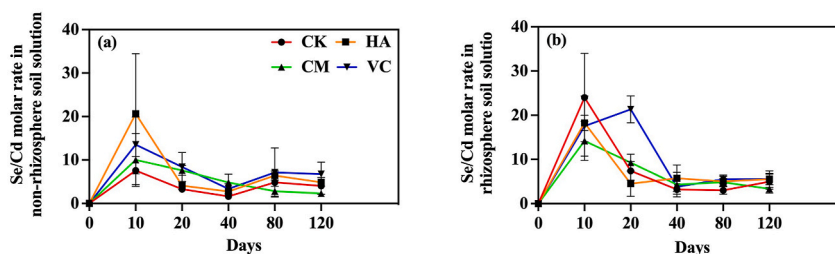


Fig. 5. Dynamics of Se/Cd molar ratios in soil solutions of non-rhizosphere (a) and rhizosphere (b) samples induced by different types of exogenous OM. Values were recorded as mean \pm SD, $n = 3$.

3.5. Species of Se and Cd in soil

In non-rhizosphere, compared to CK treatment, HA application increased the proportion of HA-bound Se, but VC decreased the proportion of water-soluble Se ($p < 0.05$) (Fig. 6 a) (Table S8). In the rhizosphere, compared to CK treatment, HA application increased the proportion of HA-bound Se, but CM decreased the proportion of ligand-exchangeable Se ($p < 0.05$) (Fig. 6 b) (Table S8).

In non-rhizosphere, compared to CK treatment, HA application increased the proportion of residual Cd, and CM and VC increased the proportion of carbonate Cd ($p < 0.05$) (Fig. 6 c) (Table S8). In the rhizosphere, compared to CK treatment, HA application increased the proportion of residual Cd ($p < 0.05$) (Fig. 6 d) (Table S8).

3.6. Se and Cd concentrations in rice tissues

Se concentrations in rice tissues were not significantly affected by exogenous OM applications compared to CK treatments (Fig. 7 a–d; Table S9). However, the application of CM led to a significant rise in straw Se concentrations (with an increase of 54.78 %) ($p < 0.05$) (Fig. 7 b).

Compared to the CK treatments, the applications of exogenous OMs did not result in any significant changes in Cd concentrations in rice tissues (Fig. 7 e–h; Table S9). However, when HA was applied, there was a notable reduction observed in grain Cd concentrations (a decrease of 56.90 %) ($p < 0.05$) (Fig. 7 h).

Compared to the CK treatments, the applications of exogenous OMs did not result in any significant changes in Se and Cd accumulations in rice tissues (Table S10).

3.7. Se and Cd translocation in rice tissues

In contrast to CK treatments, the applications of exogenous OMs did not have a substantial effect on the translocations of Se and Cd within rice tissues (Fig. 8; Table S11). However, there was a notable increase of 91.46 % in the values of $\text{SeTF}_{\text{root-stem}}$ in rice treated with CM compared to rice treated with VC (Fig. 8 a). Additionally, the values of $\text{CdTF}_{\text{root-stem}}$ were significantly higher by 138.30 % in rice treated with HA compared to rice treated with CM and VC (Fig. 8 d).

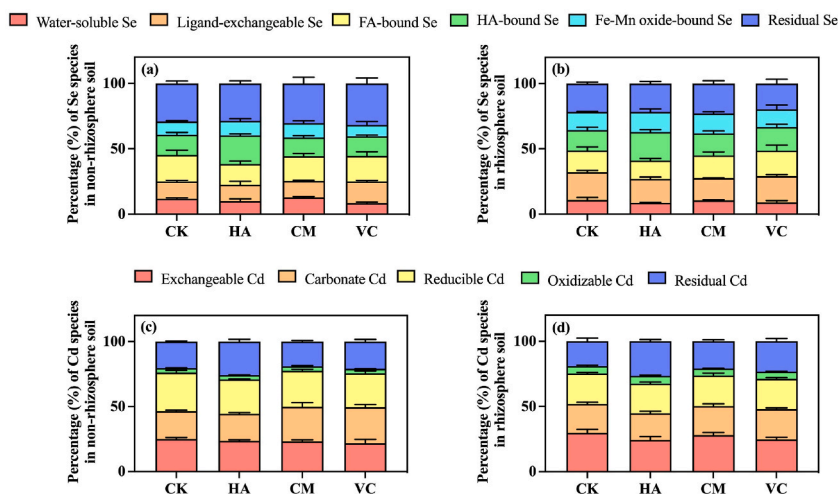


Fig. 6. Effects of exogenous OM on the percentages (%) of Se and Cd species in non-rhizosphere (a, c) and rhizosphere (b, d) soil. Values are recorded as mean \pm SD, $n = 3$.

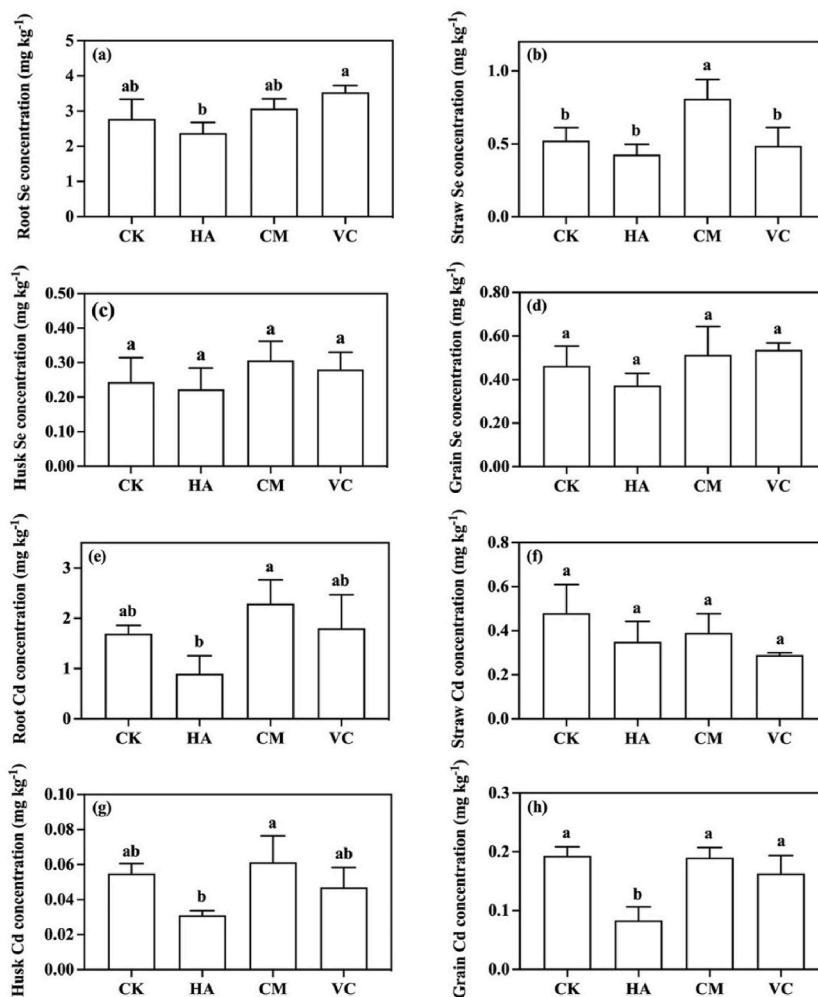


Fig. 7. Se and Cd concentrations in rice root (a, e), straw (b, f), husk (c, g), and grain (d, h) from the treatments treated with different types of exogenous OMs. Values are recorded as mean \pm SD, $n = 3$. Distinguishing lowercase letters denote notable distinctions between treatments with a significance level of $p < 0.05$ or $p < 0.01$ (one-way ANOVA with Tukey's HSD).

3.8. Relationship between Se/Cd molar ratios and the concentrations of Se and Cd

By combining the data from all treatments with/without different types of exogenous OMs, grain Se concentrations were not correlated with Se concentrations and Se/Cd molar ratios in the rhizosphere soil solution at day 80 and day 120 ($p > 0.05$) (Fig. 9a–d). Grain Cd concentrations were not correlated with Cd concentrations and Se/Cd molar ratios in the rhizosphere soil solution at day 80 and day 120 ($p > 0.05$) (Fig. 9e–h). Except, a significant positive association was observed between Cd concentrations in grains and in the rhizosphere soil solution at day 120 ($r = 0.578^*$, $p < 0.05$) (Fig. 9g).

4. Discussion

4.1. Effect of exogenous organic matters on grain yield

Increasing food production is essential to meet the food demands of a growing global population. However, from 1989 to 2008, the average annual growth rates for grain yield were merely 1.6 %, 1.0 %, 0.9 %, and 1.3 % for maize, rice, wheat, and soybean, respectively [49]. In this study, even in the absence of statistically significant changes, the application of exogenous OMs still facilitated a certain degree of improvement in rice yield by 4.55%–15.10 % (Fig. 2 d), thereby holding positive implications for food security concerns.

This enhancement of rice yield by exogenous OMs may be attributed to their promotion of rice growth and inhibition of Cd uptake in rice. According to the results of previous studies, the increase in yield of this study (Fig. 2 d) might be attributed to the exogenous OMs, which improved soil porosity and aggregation, activated growth-related signaling pathways, and regulated the physical and

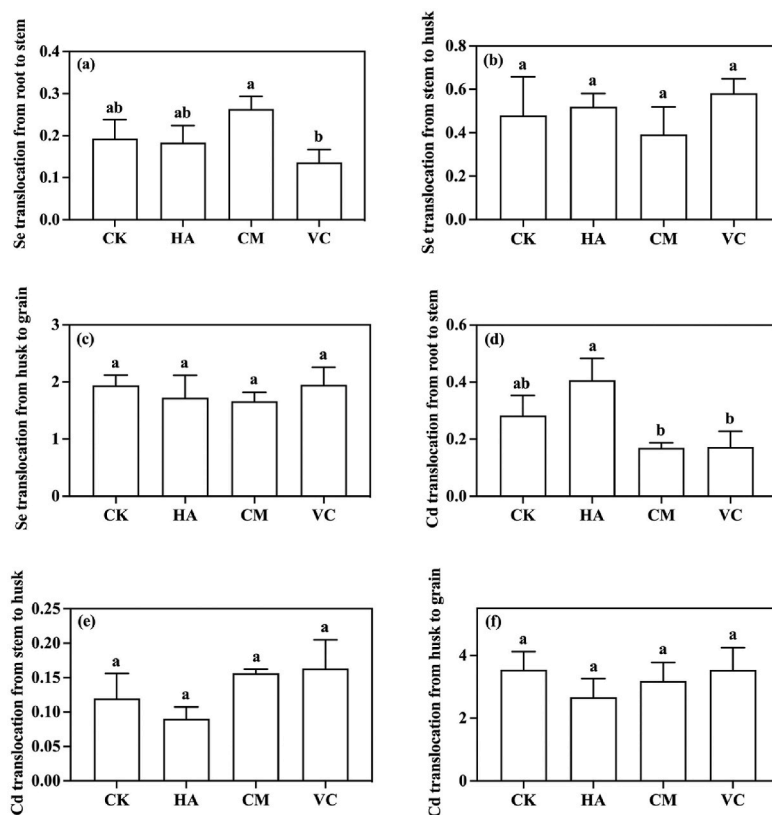


Fig. 8. Effects of exogenous OMs on Se and Cd translocations from root to stem (a, d), from stem to husk (b, e), and from husk to grain (c, f). Values are recorded as mean \pm SD, $n = 3$. Distinguishing lowercase letters denote notable distinctions between treatments with a significance level of $p < 0.05$ or $p < 0.01$ (one-way ANOVA with Tukey's HSD).

chemical properties of soils [24]. Based on the results of this study, the increase in rice yield might also be attributed to the decrease in Cd toxicity in rice facilitated by OM application. This is evident from the reduction in Cd concentration in rice grain under both treatments (HA and VC) associated with increased rice yield (Fig. 2 d; 7 h).

4.2. Se dynamics in soil solution induced by exogenous OMs

The Se concentrations in the soil solutions exhibited a rise at the beginning of the study period, spanning from day 0 to day 20, and subsequently declined in accordance with the growth period (Fig. 3c and d). Therefore, the following discussion is divided into two sections (day 0-day 20 and day 40-day 120), focusing on the rhizosphere and non-rhizosphere samples separately.

From day 0 to day 20, soil solution Se concentrations gradually increased, but the trends of these variations caused by exogenous OMs were different between the non-rhizosphere and rhizosphere samples (Fig. 3c and d). In the non-rhizosphere samples, exogenous OM elevated soil solution Se concentrations (Fig. 3c). This indicated that exogenous OMs facilitated the solubilization of some solid phases of soils, leading to the liberation and migration of Se from its fixed state in these solid phases into the solution phases of soils (Fig. 3c). We found that Se and Fe concentrations in non-rhizosphere soil solutions were significantly and positively correlated on day 10 ($r = 0.671$, $p < 0.05$) and day 20 ($r = 0.783$, $p < 0.05$), respectively (Fig. 4a and b) (Table S5). The above significantly positive correlations between Se and Fe concentrations in non-rhizosphere soil solutions indicated that the released Se mainly came from Fe-Mn oxides (Fig. 4a and b). This phenomenon might be attributed to the ability of OM to facilitate the reduction and dissolution of Fe-Mn oxides by serving as a mediator for electron transfer [21]. Consequently, this process expedited the liberation of Se from Fe-Mn oxide into the soil solution. However, the results varied in the rhizosphere (Fig. 3d). In the soil solution of the rhizosphere, there was no observed relationship between Se and Fe concentrations (Table S5). These results suggested that the fluctuations in Se concentrations within rhizosphere soil solutions remained unaffected by the dissolution of Fe-Mn oxides. This phenomenon may be attributed to the release of oxidizing substances, such as O_2 , by rice roots, which impedes the reduction-induced dissolution of Fe/Mn oxides [50]. Therefore, element concentrations in rhizosphere soil solutions might be mainly affected by the characteristics of OM [27]. HA, CM, and VC all contain high molecular weight and insoluble OMs, which accordingly decrease the solubility of Se via adsorption [28]. Meanwhile, the high molecular weight and insoluble OMs can interact with soil, resulting in the formation of insoluble aggregates that effectively trap and incorporate Se within them [28]. However, CM and VC also have low molecular weight and soluble OMs, which can enhance the solubility of Se through competition for sorption sites [26,28]. As a result, HA decreased the soil solution Se

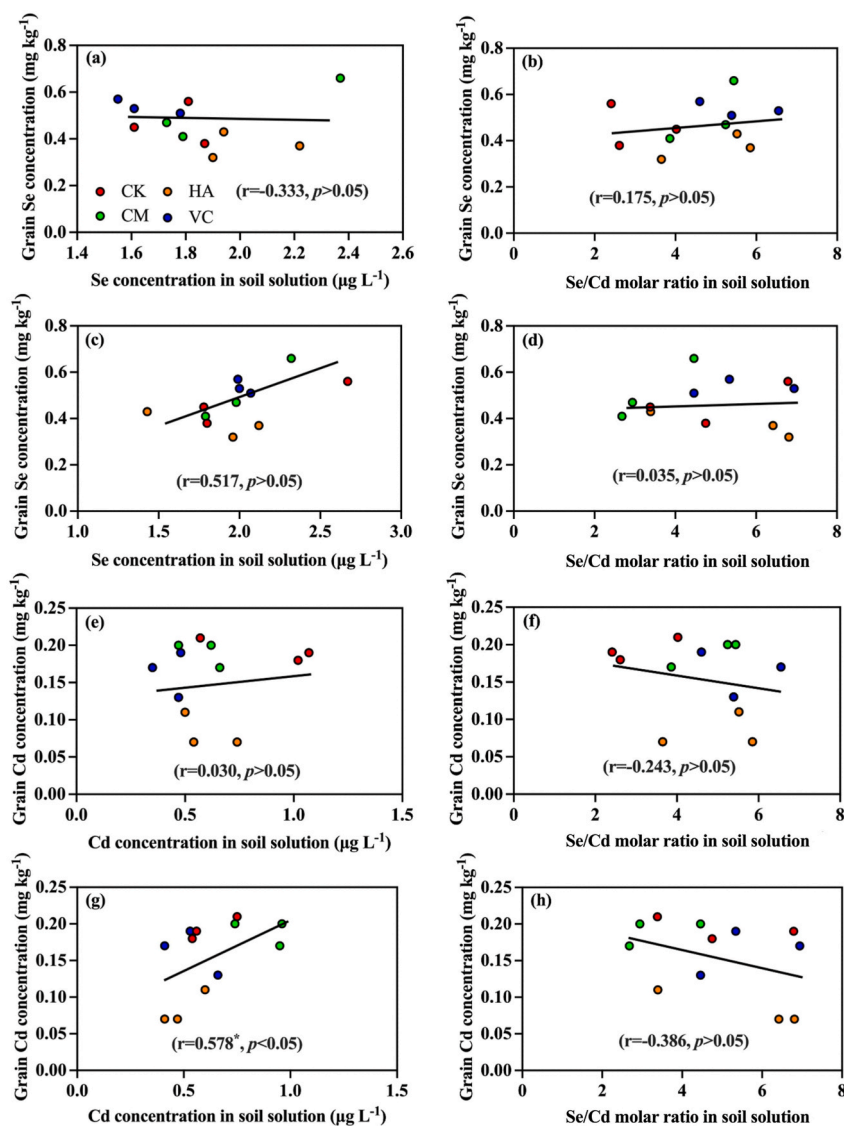


Fig. 9. Scatter diagrams. Correlations of Se (or Cd) concentrations between grains and soil solutions at day 80 (a, e) and day 120 (c, g) supplied with/without different types of OMs. Correlations between grain Se (or Cd) concentrations and Se/Cd molar ratios in soil solution at day 80 (b, f) and at day 120 (d, h) supplied with/without different types of OMs. Significance is presented as * (or **) at the $p < 0.05$ (or $p < 0.01$) level.

concentrations, whereas the other OMs (CM and VC) had lesser effects or even increased soil solution Se concentrations (Fig. 3 d). Our results showed that the trends in Se concentrations in the rhizosphere soil solution (Fig. 3 d) aligned with the trends in Se species that can be absorbed by crops in rhizosphere soils (water-soluble Se, ligand-exchangeable Se, and FA-bound Se) (Fig. 6 b), which were lower in HA treatments than in other treatments. To summarize, from day 0 to day 20, Se concentrations in non-rhizosphere soil solution were released from Fe-Mn oxides and promoted by exogenous OMs, while rhizosphere soil solution Se concentrations might be controlled by the characteristics of exogenous OMs.

From day 40 to day 120, Se concentrations in soil solutions decreased to a similar and stable level, regardless of whether they were non-rhizosphere or rhizosphere samples (Fig. 3c and d). Correlation analysis (all soil solutions) revealed that the variations in Fe concentrations or pH, along with the addition of exogenous OMs, did not have an impact on Se levels (Table S5). Previous studies showed that OM application usually resulted in low inhibition or even promoting effects on Se bio-availability in waterlogged soils [21, 51–54]. Therefore, the waterlogged environment in this study may be responsible for soil solution Se concentrations that are not influenced by OMs (Fig. 3c and d). Our study showed that in waterlogged conditions, the effect of OM application, particularly HA, on Se bioavailability in soil may be attributed to an additional potential mechanism. Under waterlogged conditions, HA can directly bind to Se and form HA-bound Se to reduce Se bioavailability in soil (Fig. 6a and b). At the same time, HA application also increased the pH of the soil solution (Fig. 3a and b), and accordingly increased Se bioavailability in the soil through promoting the dissolution of HA-bound Se. Therefore, we hypothesize that the absence of a significant impact of HA on Se concentration in the soil solution from

day 40 to day 120 may be attributed to the synergistic contributions of HA application's inhibitory effects on Se bioavailability through complexation and promotional effects on Se bioavailability through increasing soil solution pH.

4.3. Cd dynamics in soil solution induced by exogenous OMs

Compared to the CK treatments, the applications of HA and VC inhibited soil solution Cd concentrations, while the CM application initially elevated, then inhibited, and finally elevated Cd concentrations, in both the non-rhizosphere and rhizosphere samples (Fig. 3e and f). Therefore, we focus on the rhizosphere in the following analysis.

HA, as a stable and high molecular weight OM, has the potential to reduce Cd concentrations in soil solutions by inhibiting soil Cd bio-availability through bounding Cd ions to the solid phase of soil [27]. Our study showed that soil solution Cd concentrations were reduced by the application of HA (Fig. 3f). This might be explained by the diminution in the proportions of soil-exchangeable Cd (easily migrates into the solution phase) and the increase in the proportions of residual Cd (hard migrates into the solution phase) [47] (Fig. 6d). In contrast, in addition to containing high molecular weight OM, VC and CM also have low molecular weight OMs, such as formic acid, acetic acid, and fulvic acid [26,28]. These low molecular weight OMs, by using their functional groups, engaged in a competitive interaction with Cd ions to occupy the adsorption sites on soil surface and facilitated the solubilization of Cd ions adsorbed on the soil surface [24,26,39,55]. Therefore, the ability of VC and CM to reduce the concentration of Cd in soil solution was similar or weaker than that of HA (Fig. 3e and f). Additionally, the change in pH typically influences the bioavailability of Cd [27]. Our findings indicate that compared to CM and VC, HA elevates soil solution pH (Fig. 3a and b), potentially facilitating the transformation of exchangeable Cd into residual Cd (Fig. 6c and d), and subsequently reducing its concentration in the soil solution (Fig. 3e and f). Additionally, bands were observed in the protein-specific spectral region (amide I), with the main peak at about 1640 cm^{-1} , corresponding to the asymmetric stretching of the carboxyl group in the FTIR spectra of VC (1648.27 cm^{-1}) and CM (1639.70 cm^{-1}) but not HA (Figs. S1–S3). This indicates that compared to HA, CM and VC have much more protein [41]. Therefore, HA was more effective than CM and VC in reducing Cd in soil solution because proteins in CM and VC were more easily decomposed [28]. Consequently, the variation in soil Cd concentration may be attributed to the combined effect of the complexation of OM and Cd, the change of soil pH induced by OMs, and the constituents of OM.

4.4. Se:Cd molar ratio dynamics in soil solution induced by exogenous OMs

The bio-availabilities of Se and Cd in soils were thought to be influenced by Se/Cd molar ratios. This phenomenon occurred due to the reaction between Se and Cd, resulting in the synthesis of a Cd-Se complex, which could not easily be taken up by crops [9,30,56]. Specifically, it has been observed that the maximum percentage decrease in Cd uptake induced by Se is generally obtained at Se/Cd molar ratios equal to or close to 1 [30]. However, the Se/Cd molar ratios in the soil solution ranged from 1.60 to 20.63 (mean 6.34) in non-rhizosphere and 3.01 to 23.98 (mean 8.57) in rhizosphere samples (Fig. 5a and b), which values were much higher than 1. Hence, it was plausible to suggest that the alterations in Se/Cd molar ratios observed in this study may not be the primary determinant influencing grain Cd concentrations.

4.5. Se and Cd uptake by rice plants and its relationship with exogenous OMs

The levels of Se and Cd in rice grains (Fig. 7d and h) were predominantly determined by the bioavailability of these elements present in the soil (including their concentrations and molar ratios within the rhizosphere soil solution), along with their subsequent translocations within different rice tissues [30,56]. We now discuss the relative importance of different influencing factors.

First, the correlation between the Se-Cd bio-availabilities in rhizosphere soil and their concentrations in rice grains was studied by combining the data from all treatments. We explored the relationship between Se and Cd concentrations in soil solutions collected at day 80 and day 120 and those in rice. The results indicated no correlation in the Se concentrations between grain and rhizosphere soil solution at day 80 ($r = -0.333, p > 0.05$) (Fig. 9a) but a slight positive correlation at day 120 ($r = 0.517, p > 0.05$) (Fig. 9c). In addition, our analysis revealed no significant correlation between grain Se concentrations and Se/Cd molar ratios in rhizosphere soil solution at either day 80 ($r = 0.175, p > 0.05$) (Fig. 9b) or day 120 ($r = 0.035, p > 0.05$) (Fig. 9d). The abovementioned correlation coefficient values might suggest that the Se concentrations in grains were primarily influenced by the levels of Se in the soil solution, rather than the variety of molar ratios of Se to Cd in the soil solution, especially at day 120 (Fig. 9c–d). For the uptake of Cd, the correlations between Cd concentrations in the grains and in the rhizosphere soil solutions were found to be no correlation at day 80 ($r = 0.030, p > 0.05$) (Fig. 9e) but significantly positive at day 120 ($r = 0.578^*, p < 0.05$) (Fig. 9g). Meanwhile, there was no correlation between grain Cd concentrations and Se/Cd molar ratios in the rhizosphere soil solution at day 80 ($r = -0.243, p > 0.05$) (Fig. 9f) but a slight negative correlation at day 120 ($r = -0.386, p > 0.05$) (Fig. 9h). The correlation coefficients might suggest that the levels of Cd in grains were more influenced by soil Cd concentrations, rather than the molar ratios of Se to Cd in the soil solution, especially at day 120. Hence, the existence of OMs could potentially influence the levels and molar ratios of Se and Cd in the soil solution from day 40 to day 120 except the levels of Se in the soil solution. Based on the statistical analysis above, it is more probable that the concentrations of Se and Cd in rice are influenced by their respective concentrations in the soil solution due to the presence of OMs.

Second, we examined the correlations between the Se (or Cd) translocations in rice tissues and their concentrations in rice grains. OM applications did not have a significant impact on the Se (or Cd) translocation factors in rice tissues (Fig. 8). This suggested that the Se (or Cd) translocation processes in rice tissues were not affected by OM applications. Therefore, it might be concluded that the influence of exogenous OMs on grain Se and Cd concentrations was not attributed to their control over the translocation of these

elements within rice tissues.

Third, we investigated the impact of OM on the growth of rice plants to determine if alterations in Se and Cd levels in rice were influenced by the variations in rice biomass. Our findings indicated that the applications of OMs did not have significant impacts on the biomass of rice (Fig. 2) and the accumulations of Se and Cd in rice tissues (Table S10). Therefore, it is possible that the alterations in Se and Cd concentrations observed in rice tissues were not primarily influenced by the changes in rice biomass.

To summarize, the results indicated that the levels of Se and Cd in grains might be primarily influenced by their concentrations in the rhizosphere soil solution, rather than by the molar ratios of Se/Cd in the rhizosphere soil solution, or the translocations and accumulations of Se and Cd within rice tissues.

4.6. Mechanism to stabilize Se uptake in rice while minimizing Cd accumulation

The natural presence of elevated levels of both Se and Cd in soils can result in excessive accumulation of Cd in Se-enriched rice [9, 57]. Hence, it is crucial to investigate and devise strategies that effectively reduce Cd uptake in rice while ensuring minimal interference with Se uptake. Our study found that the application of exogenous OMs (HA, CM, and VC) did not significantly affect the concentration of Se in soil solution from day 80 to day 120 (Fig. 3c and d). The waterlogged environment in this study may be responsible for soil solution Se concentrations that are not influenced by OMs [21,51–54]. Our study showed that in waterlogged conditions, the effect of OM application, particularly HA, on Se bioavailability in soil may be attributed to an additional potential mechanism. Under waterlogged conditions, HA application decreased Se bioavailability in soil by increasing HA-bound Se (Fig. 6a and b). However, this process also increased the pH of the soil solution (Fig. 3a and b) and promoted Se bioavailability in the soil by promoting the dissolution of HA-bound Se. Therefore, the absence of a significant impact of HA on Se concentration in the soil solution (Fig. 3c and d) may be attributed to the synergistic contributions of HA application's inhibitory effects on Se bioavailability through complexation and promotional effects on Se bioavailability through increasing soil solution pH.

In terms of Cd, the Cd concentrations in soil solutions can be affected by OM application through complexation. Recalcitrant macromolecules in OM internally immobilize Cd through complexation, thereby reducing Cd concentration in soil solution [27]. However, unlike humic acid (HA), both CM and VC also contain low molecular weight OMs such as formic acid, acetic acid, and fulvic acid [24,26,39,55]. These small molecular weight organic compounds compete with Cd ions through their functional groups and occupy adsorption sites on the soil surface, promoting the desorption process of Cd ion adsorption on the soil surface. These low molecular weight organic compounds resulted in similar or less effective inhibition effects of VC and CM on Cd bioavailability compared to that of HA [27,29,39], which is the same in this study (Fig. 3e and f; 6c, d). In addition, in comparison to VC and CM, HA does not have C=O functional groups (Figs. S1–S3), suggesting that VC and CM exhibit higher protein content [41]. The protein in VC and CM are more easily to be decomposition to low molecular weight OMs than HA, which makes HA more effective than CM and VC in decreasing Cd bioavailability in soil [28]. Furthermore, compared to CM and VC, HA decreases Cd bioavailability in soil (Fig. 6c and d) by elevating the pH of the soil solution (Fig. 3a and b). Consequently, HA is more effective than CM and VC in reducing Cd uptake in rice due to the more stable component of HA and higher soil solution pH induced by HA.

5. Conclusions

The stable cultivation of Se-rich and Cd-low rice in Se-Cd-rich paddy fields is a critical challenge in advancing green Se-rich agriculture. The goal is to reduce Cd concentration in rice grain without affecting Se concentration. This study investigated the effects of different OMs (HA, CM, and VC) on Se and Cd concentrations in rice tissues through pot experiments. The results showed that OMs have no significant effect on Se and Cd concentrations in rice grains, except that Cd concentrations in rice grains were significantly decreased by HA application. This effect is attributed to the influence of exogenous OMs on the concentrations of Se and Cd in soil solution, rather than on the Se/Cd molar ratios in soil solution or their translocation and accumulation within rice tissues. HA decreased Se and Cd bioavailability in soil by increasing HA-bound Se and residual Cd, respectively. Meanwhile, HA increased soil solution pH, which was negative for Cd bioavailability through bounding Cd to soil solid phase but positive for Se bioavailability through dissolving HA-bound Se. This additive effect made HA lowered Cd concentration more than Se concentration in both soil solution and grain. This property enables the utilization of HA in Se-Cd-rich paddy soils to mitigate rice's Cd uptake as much as possible while exerting a small impact on Se uptake in rice. The additive effect observed in HA was not found in CM and VC, which makes CM and VC applications do not significantly affect Se and Cd bioavailability in soil and in rice grains. Notably, although VC did not significantly affect the concentrations of Se and Cd in rice grains, there was a 15.83 % increase in Se concentration and a 15.52 % decrease in Cd concentration. The relative variation between them reached 31.35 %. This property makes VC suitable for use in natural Se-Cd-rich paddy soils with less Cd contamination to maximize the Se concentration in rice. Further studies are needed to determine the optimal selection of these OMs based on specific Cd contamination levels. In summary, this study elucidates how exogenous OMs impact the absorption of Se and Cd in rice grown in natural Se-Cd-rich paddy soil. The findings provide valuable theoretical and practical insights for producing Se-enriched rice with maximum safety.

Funding

This work has been jointly supported in part by Joint Funds of the National Natural Science Foundation of China (Program No. U21A2023), Natural Science Basic Research Program of Shaanxi (Program No. 2022JQ-252), Pre-research Project Funding of Hebei GEO University (Program No. KY2024QN15), Key Laboratory of Se-enriched Products Development and Quality Control, Ministry of

Agriculture and Rural Affairs/National-Local Joint Engineering Laboratory of Se-enriched Food Development (Grant No. Se-2021C02), Natural Science Foundation of China (Grant No. 41872250), Special Fund for Technological Innovation guidance of Shaanxi Province (2022) - Regional Innovation Capacity Guidance Plan (Grant No. 2022QFY09-02).

CRedit authorship contribution statement

Hongyu Zhang: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Shuyun Xie:** Writing – review & editing, Supervision, Project administration. **Xiaoping Du:** Visualization, Investigation. **Zhengyu Bao:** Writing – review & editing, Investigation. **Feng Xu:** Investigation, Formal analysis. **Salah Fadlallah Awadelseid:** Writing – review & editing, Investigation. **Oraphan Yaisamut:** Writing – review & editing, Formal analysis.

Declaration of competing interest

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.

Acknowledgments

The authors also appreciate the constructive comments and suggestions of the anonymous reviewers and journal editors for editorial handling.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e37740>.

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