



Effects of selenium on biogeochemical cycles of cadmium in rice from flooded paddy soil systems in the alluvial Indus Valley of Pakistan

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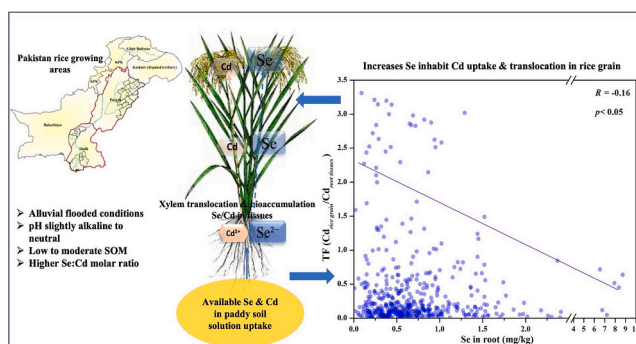
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HIGHLIGHTS

- Effects of Se on Cd geochemical cycle alluvial flooded soil-rice system in Pakistan reported first time
- Geographically weighted regression modeling revealed great spatial nonstationarity.
- The translocation factor and a negative correlation affirm that Se inhibits the translocation of Cd to rice grains.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Elena Paoletti

Keywords:

Selenium-cadmium
Geographically weighted regression
Interaction-translocation
Paddy soil-rice
Pakistan

ABSTRACT

This study delves into the pollution status, assesses the effects of Se on Cd biogeochemical pathways, and explores their interactions in nutrient-rich paddy soil-rice ecosystems through 500 soil-rice samples in Pakistan. The results showed that 99.6 % and 12.8 % of soil samples exceeded the World Health Organization (WHO) allowable Se and Cd levels (7 and 0.35 mg/kg). In comparison, 23 % and 6 % of the grain samples exceeded WHO's allowable Se and Cd levels (0.3 and 0.2 mg/kg), respectively. Geographically Weighted Regression (GWR) model results further revealed spatial nonstationarity, confirming diverse associations between dependent variables (Se and Cd in rice grain) and independent variables from paddy soil and plant tissues (root and shoot), such as Soil Organic Matter (SOM), pH, Se, and Cd concentrations. High Se:Cd molar ratios (>1) and a negative correlation ($r = -0.16$, $p < 0.01$) between the Cd translocation factor (Cd in rice grain/Cd in root) and Se in roots suggest that increased root Se levels inhibit the transfer of Cd from roots to grains. The inverse correlation between Se and Cd in paddy grains was further characterized as Se deficiency, no risk, high Cd risk, Se risk, Cd risk, and Se–Cd co-exposure risk. There was no apparent risk for human co-consumption in 42.6 % of

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<https://doi.org/10.1016/j.scitotenv.2023.168896>

Received 3 September 2023; Received in revised form 24 November 2023; Accepted 24 November 2023

Available online 1 December 2023

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grain samples with moderate Se and low Cd. The remaining categories indicate differing degrees of risk. In the study area, 31 % and 20 % of grain samples with low Se and Cd indicate Se deficiency and risk, respectively. High Se and low Cd levels in rice samples suggest a potential hazard for severe Se exposure due to frequent rice consumption. This study not only systematically evaluates the pollution status of paddy-soil systems in Pakistan but also provides a reference to thoroughly contemplate the development of a scientific approach for evaluating human risks and the potential dangers associated with paddy soils and rice, specifically in regions characterized by low Se and low Cd concentrations, as well as those with moderate Se and high Cd concentrations.

Synopsis: This study is significant for understanding the effects of Se on Cd geochemical cycles and their interactions in paddy soil systems in Pakistan.

1. Introduction

Selenium (Se) is a crucial trace element for humans and plays an important role in regulating physiological processes, fostering cardiovascular health, minimizing metal toxicity, and reducing cancer risk by mitigating oxidative stress (Chauhan et al., 2019), which can lead to specific diseases such as Kashin–Beck and Keshan in particular regions when a daily intake below 0.127 μg (Lin et al., 2012). However, excessive Se intake can result in toxicity, leading to neurological and skin issues (Rayman, 2012; Zwolak and Zaporowska, 2012). Human Se intake by food, such as low or high rice, is closely tied to the Se content in the soil (Dinh et al., 2018). This link is significant due to the soil's role as a major Se source for crops and forages, which form a substantial part of the human diet (Gupta and Gupta, 2000). Selenium enters the soil through natural processes like rock weathering and human activities such as agriculture, irrigation, and industrial emissions (Dhillon and Dhillon, 2019).

Globally, soil Se concentration varies, with high levels in North America and Western Europe, such as France, resulting in Se intake ranging from 93 to 134 $\mu\text{g}/\text{day}$ (Duntas and Benvenega, 2015; Stoffaneller and Morse, 2015). In contrast, China faces a Se deficiency in 72 % of its soil, affecting 700 million people with an average intake of 28 to 40 $\mu\text{g}/\text{day}$, falling below the recommended daily intake for adults (Gao et al., 2011; Huang et al., 2017; Lin et al., 2012). Previous publications demonstrated that the rice plant is notable for accumulating Se, particularly in grains, the edible part of the plant (Deng et al., 2017; Zhou et al., 2020). The uptake and accumulation of Se in rice plants raise concerns about deficiency or crop toxicity (Dinh et al., 2019). Various factors, including soil pH, organic matter, and chemical interactions, influence Se availability (Z. Li et al., 2017). Herein, understanding the biogeochemical cycle of Se in the context of a soil-rice system is crucial for managing Se levels in rice crops and addressing potential environmental and health risks.

Cadmium (Cd) is nonessential and the most toxic and widespread trace element in agricultural soils (Rahman and Singh, 2019). Due to its relatively high toxicity and plant availability, it poses a direct contact risk to humans and terrestrial ecological receptors (Lin et al., 2012). Cadmium exposure triggers severe human health problems, such as bone disease, lymphocytosis, kidney damage, hypertension, and cancer (Dong et al., 2021). As a result of natural and anthropogenic impacts, some countries, e.g., China, India, Malaysia, Australia, Thailand, and the USA, face soil Cd pollution (Ali et al., 2020; Arao et al., 2010; Dong et al., 2021; Loganathan et al., 2012). Because of its strong mobility in the soil-plant system, Cd can efficiently translocate and bioaccumulate in plants, particularly in rice crop systems (Hu et al., 2020). The severe toxicity of Cd can adversely disturb the natural growth of cultivated crops, most importantly causing physiological metabolism disorders and decreasing the quality of agricultural products (Ismael et al., 2019). Cadmium can be efficiently translocated and bioaccumulated in rice crop systems, primarily in grains (Hu et al., 2016). Over 60 % of the population worldwide consumes rice as a staple food; hence, rice is the primary source of Cd intake (Song et al., 2017; P. Wang et al., 2019). Consequently, Cd pollution in paddy soil-rice systems presents a considerable hazard to food stability.

Recently, several studies proposed that Se showed antagonistic effects on potentially toxic elements such as mercury (Hg), arsenic (As), antimony (Sb), and Cd in paddy soil-rice systems (Chang et al., 2020; Ding et al., 2015; Kumar et al., 2014; Wan et al., 2016). The effect of Se on plants under Cd stress has been extensively studied at the biochemical level. For instance, Lin et al. discovered that the external Se supplement significantly reduced the Cd levels in rice by activating protective mechanisms against oxidative stress (Lin et al., 2012). Saidi et al. reported that the Se protective function is associated with reduced lipid peroxidation, enhanced scavenging of reactive oxygen species, and decreased Cd uptake, transport, and distribution in plant tissues (Saidi et al., 2014). In contrast, Huang et al. suggested that exogenous Se reduces Cd translocation and accumulation, with Cd affecting Se uptake but not its translocation within the plant (Huang et al., 2017). Ma et al. investigated Si-Cd interactions at the cellular level using rice suspension cells, discovering that hemicellulose-bound Silicon (Si) in cell walls acts as a barrier, hindering Cd uptake. These studies indicated that Cd's uptake, translocation, accumulation, and toxicity to paddy plants could be impeded or supported by Se fertilization, i.e., Se application or foliar spray. However, investigations and understanding of the interaction between Se and Cd in natural paddy environments are lacking, particularly in regions suffering from Se and Cd contamination.

Pakistan, the main rice-producing country globally, produces an average of 6.8 million tons of rice annually and exports 8.2 % of the world's rice (Aslam et al., 2020). To date, no study has investigated the geochemical cycle interactions, effects, and pollution risks of Se and Cd in Pakistan's paddy soil-rice system. Therefore, the goals of this comprehensive study are to examine the status of Se and Cd contamination and the effects of Se on the biogeochemical cycles of Cd accumulation in rice grains from flooded paddy soil systems in the alluvial Indus valley of Pakistan; to assess the relationship between independent rice grain Se and Cd concentrations and the most significant independent paddy soil and plant tissue (root and shoot) properties, i.e., pH, SOM, Se, and Cd concentrations through GWR modeling; to investigate the interactions and effects between Se and Cd and their transportation among and bioaccumulation in natural sites; and to examine Se and Cd risk classification based on Se and Cd concentrations in rice grains to provide further insights into the state of the environment.

The significance of this study will help assess pollution levels, identify risk areas, and understand the Se-Cd distribution and accumulation in Pakistan's paddy soil-rice system. This research supports the development of regional and national Se and Cd mitigation measures, enabling better practices to address Se deficiency in the food chain and control Se and Cd release in soils. Moreover, it fills gaps in environmental research in Pakistan.

2. Materials and methods

2.1. Research area

The Arabian Sea coastline belt in South Asia encircles Pakistan. It encompasses 137 administrative districts and covers an area of approximately 796,095 km^2 . About one-third of Pakistan's land area is desert, while the rest mainly comprises agricultural terrains and low-

lying regions (Ali et al., 2022). The climate is largely semiarid to arid, in contrast to the more temperate conditions in the northwest (Ali et al., 2019). The Hindu Kush, Karakoram, and Himalaya Mount ranges are in the north zone; Kashmir high mountains (contested territory) are in the northwest (Fig. S1), and the Baluchistan Highland region is in the western part. Flat, low-lying Indus Flood Plain surrounds the country's agricultural lands, the Quaternary alluvial sediments, and condensed organic matter (OM) in soils. Broad riverine systems, *i.e.*, Ravi, Jhelum, Sutlej, Chenab, and Indus rivers, are in the northwest zone. Wind-blown barren regions remain primarily prevalent in the adjacent areas, but permeable gravels in a restricted size range are set up in the northwest parts. Due to abundant water resources and fertile land, the alluvial region of the Indus Flood Plain, primarily situated in Pakistan's Punjab and Sindh provinces, contains extensive areas dedicated to paddy and other types of agriculture (Ali et al., 2022).

2.2. Sample collection

The entire study area comprises the alluvial Indus flood zone of the major riverine system (Indus, Ravi, Jhelum, Chenab, and Sutlej), covering almost 6000 km². The soils in the research area are a mix of sandy, loamy, and clayey textures, with loose gravel around 300 m below the surface in Quaternary alluvial deposits. They have a slightly alkaline to neutral pH and are known for low organic matter in the alluvial Indus Valley (Ali et al., 2022; Husain et al., 2012). During the harvesting period, from paddy fields, approximately 6 to 8 km² between plots was maintained, 500 plant tissues, including rice grains, shoots, and roots. The rhizosphere soil samples were randomly composed of a

total of twenty-four districts specialized in rice cultivation across two provinces in Pakistan: specifically, 86 samples from Sindh and 414 from Punjab (Fig. 1). The study area of Sindh's eight paddy-growing sampled districts covered Tando Mohammad Khan, Shikarpur, Tando Allahyar, Badin, Dadu, Thatta, Hyderabad, and Larkana. Punjab's sixteen paddy-producing sampled districts included Bahawalnagar, Faisalabad, Minda Bahauddin, Chinoat, Okara, Gujranwala, Pakpattan, Kasur, Sialkot, Sahiwal, Gujrat, Nankana Sahib, Lahore Hafizabad, Narowal, and Sheikhupura.

The mixed sample is less than five sub-samples containing 10 to 15 m of each 1 to 10 cm depth of a specific sampling site. Each plant-sub sample was first uprooted at each location and thoroughly washed away with the paddy field irrigation water. After washing, each sub-sample was integrated into composite samples wrapped in three straining mesh polyethylene zipper bags. Concurrently, plant tissue samples were again washed away with 18.2 MΩ water (Milli-Q® Integral system) to avoid cross-contamination, especially the root samples, to remove securely attached soil and samples separated to (root, shoot, and grain); they were securely wrapped in three straining mesh polyethylene bags and air-dried for several days. Before the other process, all samples were oven-dried at 35 °C to make sure samples dried completely.

The grain samples were further separated into husk, brane, and polished rice. Subsequent plant tissue samples (root and shoot) and polished grain rice were powdered using milling machinery (IKA®A11 basic-analytical grinder). All powdered plant tissues and polished rice grain samples were carefully and securely wrapped in polyethylene zipper bags and stored. Before analysis, the soil samples were at room

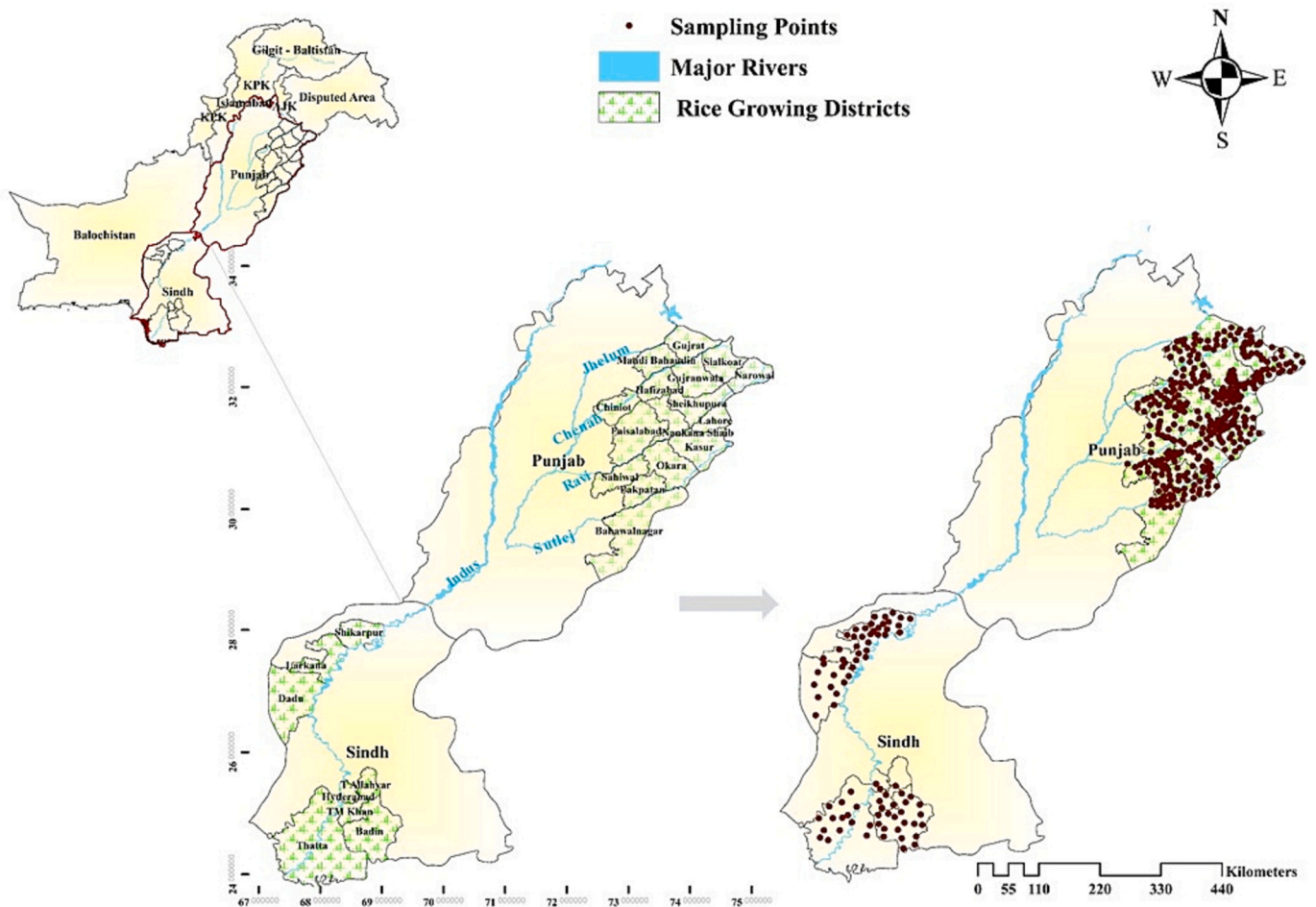


Fig. 1. Alluvial flooded paddy growing districts in the Indus Valley, with the spatial distribution of the 500 data points collected in Sindh and Punjab, Pakistan, shown.

temperature, first air-dried for several days, and then each sample was divided into two sub-samples. The sub-samples first part crashed using an agate mill and then passed through with a (2.0 mm mesh nylon sieve) for soil pH and EC measurement. Meanwhile, the second part of the sub-sample ground and passed (100 mm mesh nylon sieve) for the chemical analysis of Se and Cd. After the process of soil samples, each sample was securely wrapped in a polyethylene zipper and stored. Shortly after, all samples were transported to the Institute of Geochemistry, Chinese Academy of Sciences (IGCAS), Guiyang, China, for further chemical analysis.

2.3. Chemical analysis

All plant tissues, paddy soil, and chemical properties (SOM, pH, and EC) analysis were done at the IGCAS laboratory. The paddy soil pH and EC 4 g of individual samples were weighed and placed in polytetrafluoroethylene (15 mL PTFE, Teflon) bombs. Later, 10 mL 18.2 MΩ water was added and shaken, and measurement was done using (PHEP-EC-N sensor Hanna, Mauritius, per precision 0.001 pH). In contrast, SOM measurements were done using the potassium bichromate wet combustion technique (Wu et al., 2010). For Cd measurement in paddy soil, 50 mg of each sample (100 mesh) was placed into a 50 mL Teflon bomb. Next, 0.5 mL high-grade ultrapure hydrofluoric acid (HF, 57 M) and 3 mL nitric acid (HNO₃) were added to each bomb and put into a container (stainless-steel) and tightly closed, then put into an oven at 150 °C for 48 h. For Cd measurement in plant tissues (root, shoot, and grain), 100 g of samples were put into a bomb, and then 3 mL of HNO₃ was added to each bomb and put into an oven at 150 °C for 7 h. After that, they were removed from containers, and 1 mL of H₂O₂ (30 % v/v) and 1 mL of HNO₃ were put into each bomb, placed on a hotplate, and heated at 110 °C till the solution nearly evaporated. Next, HNO₃ 2 mL and 18.2 MΩ 3 mL of water were put into every bomb, placed in a container, and placed in an oven at 150 °C for 7 h. After 7 h, all bombs were removed from containers, and 18.2 MΩ 5 mL of water was added to every bomb and filtered through with a 0.45 μm Whatman membrane, and the solution was shifted to a 20 mL tube and sealed. After All, from each processed sample solution, a 1 mL solution was taken and diluted with 9 mL of 18.2 MΩ water, shifted to a 20 mL tube, capped, and then stored under cool conditions (up to 4 °C). After all, samples shifted to ICP-MS (ICP-MS, Agilent 77,009, California, USA) research laboratory for the Cd assessment.

The Se concentration in plant tissue (grains, shoots, and roots) was analysed through a hydride generation-atomic fluorescence spectrometry (LC-AFS 9700, BJHG, China) (Zhang, 2014). Briefly, 50 and 100 mg of each paddy soil (100 mesh) and plant tissue (powdered) sample was weighed and put in a bomb with 3 mL of ultrapure concentrated HNO₃ (16 mol/L) and 0.5 mL of ultrapure HF for samples, then tightly sealed and heated in an oven at 150 °C for 48 and 7 h, respectively. Afterward, the samples were cooled and supplemented with 1 mL of H₂O₂, and the bomb was heated on the hotplate at 110 °C for 1 h. Then, the remaining solution was supplemented with 3 mL of ultrapure concentrated HCl (6 mol/L) and heated at 100 °C for 2 h. Lastly, the final solution diluted with the 18.2 MΩ 10 mL water, and samples were taken were for analysis.

2.4. Quality control (QC) and quality assurance (QA)

For the QC and QA, the sample method blanks, replicates, standard reference materials (1640a, USA), and certified reference materials for the paddy soil and plant tissue samples (GSS-5, GSB-11, and GBWE 100359) deliberate after every 10th sample subsequently during sample preparation and chemical examination. In addition, all sample uncertainties and limitations were controlled and observed, applying regular laboratory replicates of all samples and confirming the instruments' accuracy and measurement throughout regular runs with standard solutions. During the Cd measurements in all samples, the

recoveries of related analytes were measured varying between (98.2 and 105.5 %), and the standard reference materials 1640a recovery was calculated to vary between (89.5 and 112.9 %). All replicate samples' active standard deviation was within 10 %. Se measurements in all samples, the recoveries ranging between 89.2 % to 104.6 % for the certified reference materials, and the relative standard deviation of sample replicate was within 10 %.

2.5. Geographically weighted regression (GWR) modeling

The GWR modeling determined the relationships between the dependent rice grain Se and Cd concentrations and independent soil and plant tissue variables root and shoot (SOM, pH, EC, Se, and Cd concentrations). Accordingly, a GWR model among these variables (dependent and independent) was established as follows (Fotheringham et al., 2003; Qu et al., 2015):

$$Y(u) = \beta_0(u) + \beta_1(u)\chi_1(u) + \beta_2(u)\chi_2(u) + \beta_3(u)\chi_3(u) + \beta_4(u)\chi_4(u) + \beta_5(u)\chi_5(u)\varepsilon(u) \quad (1)$$

where $Y(u)$ represents a particular vector location, $\chi_1(u)$, $\chi_2(u)$, $\chi_3(u)$, $\chi_4(u)$, and $\chi_5(u)$ represent the dependent and independent; $\varepsilon(u)$ represents the Gaussian error term at the corresponding location (u); and $\beta_0(u)$, $\beta_1(u)$, $\beta_2(u)$, $\beta_3(u)$, $\beta_4(u)$, and $\beta_5(u)$ indicate the model intercept at location u and the local regression coefficients (slopes) of the independent variables (χ_1 , χ_2 , χ_3 , χ_4 , and χ_5) respectively, at location (u). Regression coefficients at location (u) were determined by using a reweighting function as follows (Fotheringham et al., 2003; Qu et al., 2015):

$$\hat{\beta}(u) = [X^T W(u) X]^{-1} X^T W(u) Y \quad (2)$$

where Y and X signify one-dimensional arrays formed by applying the Y and X variables correspondingly, the $W(u)$ denotes the local weight matrix calculated employing a kernel function that mainly places extra emphasis on the sites in calibration locations. The sample sizes varied spatially; hence, an adaptive bi-square kernel function was applied for weight assessment. The adaptive bi-square kernel function creates a zero weight to interpretations beyond the model bandwidth, reducing their effects on the regression estimation (Bidanset and Lombard, 2014).

Moreover, verifying the presence of spatial nonstationarity is crucial to estimate whether there is substantial spatial variation in the parameter approximations from the GWR evaluation. The variability among the variable coefficients, based on a typical GWR fitted with local data and various variable coefficients, was constant (hybrid or mixed GWR) (Yang et al., 2017). We evaluated whether the specific GWR model performed better than the hybrid or mixed GWR model. Model comparison was performed with the Akaike information criterion (AIC), and in this study, we mainly assessed whether the various variable coefficients varied spatially. Thus, the ultimate adaptive bandwidth for the model was decided based on each model's values. The AIC provides a performance measure to evaluate models; a smaller AIC value specifies a best-quality model (S. Li et al., 2010). Ultimately, all data (500 sample points) were utilized to standardize the GWR model at each location; the AIC values were calculated as follows:

$$AIC = 2k - 2\ln(L) \quad (3)$$

where k signifies the number of variables and L denotes the likelihood. A model with the smallest AIC can provide the best modeling performance and greatest simplicity (Podgorski et al., 2017). The GWR 4.0 modeling program was applied to execute the GWR assessment in this research (Nakaya et al., 2014). ArcGIS 10.2 and OriginPro 8.5 were used for geostatistical calculations and creating maps and graphs.

2.6. Se and Cd bioaccumulation factors (BAFs) and translocation factor (TF)

The concentrations of trace metals absorbed by the plant tissues from the soil system were intent on BAFs. The BAFs indicate the ability of the plant tissues (root, shoot, leaves, and grains) to accumulate metal concerning those metals in the soil system (Li et al., 2014). Therefore, the BAFs of Se and Cd were assessed for every rice sample to compute bioaccumulation effects in the rice system from the uptake of Se and Cd in the paddy soil system; BAFs of every sample were calculated as follows (Li et al., 2014):

$$BAFs_{\text{tissue}} = C_{\text{tissue}} / C_{\text{paddy soil}} \quad (4)$$

where C_{tissue} is the Se or Cd concentration of paddy tissue and $C_{\text{paddy soil}}$ is the Se or Cd level of equivalent paddy soil (rhizosphere). Whereas higher BAFs value suggested more trace metals were accumulated in paddy, the BAFs > 1 specified an increased metal accumulation in plants' aerial tissues (shoot, leaves, and grain).

The TF or plant uptake factor measured the accumulation of the potential metal in the rice system (Lu et al., 2018). This TF fraction identifies the plants that translocate metals from their root system to their aerial tissues (shoots, leaves, and grains). The TF fraction of Se and Cd of every sample was calculated as follows (Li et al., 2014):

$$TF = C_{\text{tissue 1}} / C_{\text{tissue 2}} \quad (5)$$

where $C_{\text{tissue 1}}$ is the Se or Cd concentrations of rice tissue above ground and $C_{\text{tissue 2}}$ is the Se or Cd concentrations of the corresponding rice tissue (root system) underground. The TF fraction values < 1 revealed that the accumulated metal is primarily stored in the plant's system. TF > 1 indicated metal translocation to the plant's aerial tissues (shoot, leaves, and grain) (Li et al., 2014).

3. Results

3.1. Soil physicochemical factors and concentrations of Se and Cd

Overall, of the 500 data points collected, the average pH across the study area examined was 7.99 with standard deviations (± 0.36). In contrast, the average pH values in Sindh and Punjab were 7.75 (± 0.34) and 8.05 (± 0.34), respectively (Fig. S2 and Table 1). Across the entire study area, the EC was 0.87 dS/m (± 0.80). In Sindh, the soil's EC ranged from 0.67 to 7.34 dS/m, averaging 2.89 dS/m (± 1.43). In Punjab, EC levels spanned from 0.28 to 2.71 dS/m, with an average of 0.66 dS/m (± 0.30). In total SOM, the concentration stood at an average of 14.48 g/kg (± 5.76). Specifically, SOM concentrations in Sindh and Punjab were 11.61 (± 3.94) and 15.08 g/kg (± 5.89), respectively. The total Se and Cd levels in paddy soil varied from 0.18 to 40.71 and 0.002 to 2.36 mg/kg, averages 15.61 (± 3.15) and 0.28 mg/kg (± 0.18), respectively. In Sindh,

the Se and Cd concentrations in the paddy soil varied from 0.70 to 40.71 and 0.03 to 1.08 mg/kg, with averages of 14.43 (± 4.51) and 0.23 mg/kg (± 0.10), respectively. In Punjab, the Se and Cd concentrations in the paddy soil varied from 0.18 to 40.07 and 0.002 to 2.36 mg/kg, with averages of 15.86 (± 2.74) and 0.29 mg/kg (± 0.20), respectively. In Sindh, 98.84 % of paddy soil samples had Se concentrations, and 4.65 % had Cd concentrations beyond the WHO's established permissible levels of 7 mg/kg for Se and 0.35 mg/kg for Cd. Similarly, in Punjab, 98.84 % and 14.49 % of soil samples exceeded these allowable limits for Se and Cd, respectively. The high Se levels in the study area's paddy soil may result from several geological and environmental factors, particularly due to underlying geological formations (Ahmad et al., 2021). Irrigation water quality impacts paddy soil Se levels: elevated Se in irrigation water can accumulate in the soil over time (Bibi et al., 2022). Anthropogenic activities such as mining, industry, and using Se-based fertilizers and pesticides can introduce Se into the environment, elevating its concentration in soil (Ali et al., 2020).

3.2. Se and Cd concentrations in paddy tissues root, shoot, and grains

In the study areas of Sindh, the concentrations of Se and Cd in the root samples varied from 0.02 to 8.36 and 0.01 to 0.28 mg/kg, averages 0.94 (± 1.17) and 0.07 mg/kg (± 0.05), respectively; in the shoot samples, they varied from 0.01 to 4.12 and 0.001 to 0.05 mg/kg, averages 0.36 (± 0.62) and 0.02 mg/kg (± 0.01), respectively. The grain samples varied from 0.001 to 2.12 and 0.001 to 0.03 mg/kg, with averages of 0.15 (± 0.32) and 0.03 mg/kg (± 0.01), respectively. In the Punjab study area, the concentrations of Se and Cd in the root samples varied from 0.01 to 8.72 and 0.0001 to 2.89 mg/kg, with averages of 0.76 (± 0.86) and 0.24 mg/kg (± 0.28), respectively; in the shoot samples, they varied from 0.001 to 4.20 and 0.0001 to 1.52 mg/kg, with averages of 0.21 (± 0.38) and 0.06 mg/kg (± 0.12) respectively. The grain samples varied from 0.001 to 2.32 and 0.0004 to 2.24 mg/kg, with averages of 0.22 (± 0.29) and 0.06 mg/kg (± 0.19), respectively. In the research zones within Sindh, 13.9 % of rice grain samples had Se levels that surpassed the WHO permissible limit of 0.3 mg/kg. Meanwhile, in the study areas of Punjab, 7.97 % of rice samples exceeded the Se limit, and 24.15 % had Cd concentrations above the WHO's established allowable level of 0.2 mg/kg. These permissible limits are used only as references; Pakistan has not set an acceptable national limit for Se and Cd concentrations in paddy soil-rice systems.

4. Discussion

4.1. Nonstationary relationships between the dependent Se and Cd concentrations in rice grains and independent variables

The GWR is a form of local regression that assesses the spatial connections between the combined effects of independent variables on

Table 1

Se and Cd concentrations and all the variables measured in the soil-rice systems (mg/kg) sampled from the provinces of Sindh and Punjab in Pakistan.

Variables	Permissible limit	Sindh (n = 86)			Punjab (n = 414)		
		Minimum	Maximum	Average \pm std. deviation	Minimum	Maximum	Average \pm std. deviation
Grain Se	0.3	0.001	2.12	0.15 \pm 0.32	0.001	2.32	0.22 \pm 0.29
Shoot Se	–	0.01	4.12	0.36 \pm 0.62	0.001	4.20	0.21 \pm 0.38
Root Se	–	0.02	8.36	0.94 \pm 1.17	0.01	8.72	0.76 \pm 0.86
Soil Se	7	0.70	40.71	14.43 \pm 4.51	0.18	40.07	15.86 \pm 2.74
Grain Cd	0.2	0.001	0.03	0.03 \pm 0.01	0.0004	2.24	0.06 \pm 0.19
Shoot Cd	–	0.001	0.05	0.02 \pm 0.01	0.0001	1.52	0.06 \pm 0.12
Root Cd	–	0.01	0.28	0.07 \pm 0.05	0.0001	2.89	0.24 \pm 0.28
Soil Cd	0.35	0.03	1.08	0.23 \pm 0.10	0.002	2.36	0.29 \pm 0.20
pH	7–8.5	7.20	8.40	7.75 \pm 0.34	7.20	8.72	8.05 \pm 0.34
SOM g/kg	–	6.02	18.69	11.61 \pm 3.94	3.24	59.50	15.08 \pm 5.89
EC dS/m	–	0.67	7.34	2.89 \pm 1.43	0.28	2.71	0.66 \pm 0.30

Where n = number of collected samples.

dependent variables. This nonstationary correlation between metals and the soil system's influencing factors in a cropping system is done by measuring variogram-specific associations through local regression, where selected pairs of observations are averaged. The GWR modeling approach's consideration of three-dimensional sample locations enables spatial variability in variables, leading to a more accurate representation of varying correlations among selected dependent and independent variables (Ali et al., 2022). In this study, assessing spatial nonstationary is crucial for determining the extent to which constraint estimates in the GWR model exhibit significant spatial variations. Our analysis employs two variations of the GWR model: the conventional GWR model, which utilizes fixed coefficients for diverse variables, and the mixed GWR model, featuring continuously varying coefficients (Qu et al., 2014). We employed the Akaike Information Criterion (AIC) as a model selection metric to discern which model provides a more robust fit. Our findings indicate that the conventional GWR model outperforms the mixed GWR model based on AIC values. Furthermore, we assessed the spatial variability of the coefficients for diverse variables within the conventional GWR model. Based on these evaluations, we selected the optimal adaptive bandwidth for the conventional GWR model, primarily relying on AIC as a performance measure to control the bandwidth for each GWR model variant (S. Li et al., 2010).

A total of 500 data points were used to assess the regression factors, such as the model intercept, slope, and local extent of the goodness of fit (R^2), acquired by applying GWR. These regression parameters were used to determine the combined relationships between the rice grain Se and Cd concentrations (dependent variables) and the soil and paddy plant tissue properties, including pH, SOM, and Se and Cd concentrations in the soil, roots, and shoots (independent variables). The GWR mainly operates in the analytical process by moving a weighted window across the spatial dataset. Within this framework, the local slope and model intercept are estimated as the mean value of the response variable about the predictor variables. This is accomplished through a fitted point and

the corresponding dependent variable, evaluated within a distance matrix encompassing all data points (Wheeler and Páez, 2010).

Conversely, classic techniques like standard linear regression and Ordinary Least Squares (OLS) regression determine the intercept coefficient based on the assumption that the observations are independent. However, these traditional methods fall short when considering the spatial interdependence of variables over different geographical scopes (Ali et al., 2022). The R^2 is a standard local extent of the model goodness of fit (Fig. 2 a and b, and Table S1). Its value varies from (0.0 to 1.0), with higher values signifying a good fit (Comber et al., 2018). This measure of model fitness can be used to describe the amount of variance between dependent and independent selected variables (Fotheringham et al., 2003). Overall, the intercept of a model describing the relationship between a dependent (grain Se) and independent variable varied from -0.187 to 1.865 , with an average of 0.125 and a standard deviation (± 0.612). The average sum of the squared values of the dependent variables was $R^2 = 0.51$ (AIC = 71.59), and the sum of the squared residuals (SSR) was 22.066. Likewise, the overall model intercepts describing the relationship between a dependent (grain Cd) and the independent variables varied from -1.708 to 1.350 , with an average of -0.244 (0.487). However, the average R^2 was 0.604, the AIC value was 550.363, and the sum of square residuals (SSR) was 5.998.

A positive or high absolute regression coefficient value revealed positive effects and a strong correlation between variables. In comparison, a negative or low absolute regression coefficient value alluded to a negative effect and weak correlation between variables (Qu et al., 2014). The GWR findings suggested that the independent variable's influence on the accumulation of Se and Cd in rice grains fluctuated. This suggests that the relationship between the dependent and independent variables is inconsistent (spatially nonstationary) across different locations, as evidenced by the changing values of the regression coefficients. Se and Cd are usually bound to minerals and inactive SOM in the soil system, rendering them nonphytoavailable (Grüter et al., 2019; Peng et al.,

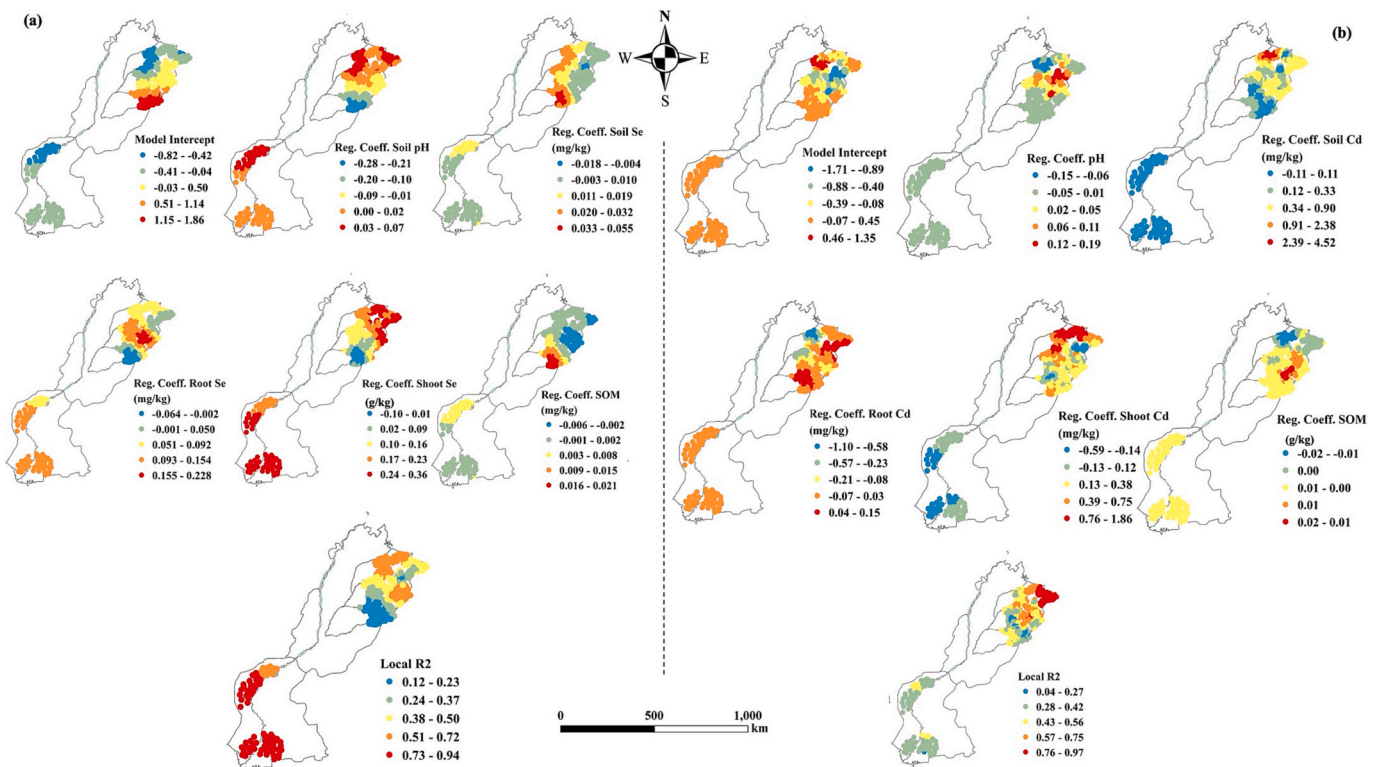


Fig. 2. Geographically weighted regression (GWR) modeling sampled from the provinces of Sindh and Punjab in Pakistan. The dependent variables included rice grain Se and Cd concentrations, and the independent variables included paddy soil properties (pH, SOM, and Se and Cd concentrations) and plant root and shoot properties (Se and Cd concentrations). The model intercept and regression coefficients of the local R^2 of the independent variables are shown (a and b).

2020). Active SOM boosts the phytoavailability of Se and Cd by raising the soil cation exchange capacity (CEC) (Hamid et al., 2019; White and Broadley, 2009). In soil solution, CEC plays a role in electron transport for the bioreduction process, which increases Se and Cd chelation and enriches nutrient solubility (Halim et al., 2020; Pezzarossa et al., 1999; D. Wang et al., 2019b). Therefore, the influence of SOM on Se and Cd phytoavailability and accumulation in plant tissues mainly relies on their concentrations and characteristics.

In plant systems, the phytoavailability of Se contrasts with the soil pH; in acidic soils, *i.e.*, low-pH conditions, soil solution adsorption decreases Se concentration, solubility, altering phytoavailability, and mobility of Se in the soil system (Guo et al., 2023). In contrast, soil solution adsorption in alkaline soils, *i.e.*, in high-pH conditions, increases Se concentration and mobility in soil systems because Se is significantly integrated into soil particles through electrostatic interfaces (Dinh et al., 2019; Guo et al., 2023). In contrast, Se was negatively correlated with the soil pH and Cd phytoavailability (Li et al., 2021). In acidic soils, there is increased desorption of Cd and weaker binding than Se; as a result, soil colloids enable increased Cd concentrations, solubility, and phytoavailability (Shaheen et al., 2013). However, in alkaline soils, Cd desorption decreases due to Cd(OH)₂ formation, lowering the Cd concentration, solubility, and phytoavailability (Gong et al., 2021). In contrast, a few studies reported that increased pH did not reduce the Cd concentration or phytoavailability in crops (Chaney, 2010; Chaney et al., 2006; Mitchell, 1997; Pepper et al., 1983). Therefore, the correlation between the chosen dependent and independent variables, as well as the effectiveness of the GWR, differed across the study areas. This is evident from the fluctuations in the local R² values corresponding to the concentrations of Se and Cd. The R² values from the GWR model vary, with values for Se ranging from 0.12 to 0.94 and those for Cd ranging from 0.04 to 0.97. The differences between the effects of each independent variable and their combination on the accumulation of Se and Cd in the rice grain were investigated. The independent variables effectively capture the accumulation of Se and Cd in the grains.

Applied adaptive bandwidth in GWR to model the correlation between Se and Cd levels in rice grains and the chosen independent variables. The model's bandwidth varies across the study area, adjusting to the local sample density at each location. A decrease in adaptive bandwidth corresponds to increased sample intensity (Propastin et al., 2008), and GWR evaluation becomes increasingly local, showing geographical characteristics similar to the products of a three-dimensional microscope (Fotheringham et al., 2003; Qu et al., 2015). Furthermore, the locations where the explanatory power of the GWR model is ineffectual are fixed by applying an adaptive kernel density assessment. The regression model is constructed by considering the ratio of locations to points within the adaptive kernel that carry a non-zero weight (DesMarais and Costa, 2019; Wheeler and Páez, 2010). Hence, the local regression incorporates further observations and the results in smoothing regions wherever the GWR model's descriptive influence is ineffectual.

4.2. Correlation between soil physicochemical factors and Se and Cd in tissues

To elucidate how various factors in paddy soil, such as concentrations of Se and Cd, pH, EC, and the amount of SOM, influence the accumulation of Se and Cd in different parts of paddy plants, including the roots, shoots, and grains, we carried out Pearson's correlation analyses to examine the relationships between soil factors in paddy fields and the concentrations of Se and Cd in paddy plant tissues. These analyses focused on paddy-growing districts in Pakistan's Sindh and Punjab provinces. Overall, the data from Pearson's correlation analyses showed a significant relationship between the Se concentrations in rice grains and those in the soil ($r = 0.447$), roots ($r = 0.564$), and shoots ($r = 0.584$), as specified in Table S2 and Fig. S3. It also revealed a weaker correlation with soil pH ($r = 0.122$), SOM ($r = 0.186$), and soil Se

negative correlation EC ($r = -0.13$). In the Sindh region, notable correlations were found: a strong positive correlation between Se in rice grains and soil Se levels ($r = 0.812$), robust correlations between Se in rice roots ($r = 0.947$) and shoots ($r = 0.950$) with soil Se levels, and negative correlations between soil Se and EC ($r = -0.17$), root Se and EC ($r = -0.167$), and grain Se and EC ($r = -0.118$), as presented in Table S3. Whereas in the Punjab region, a significant relationship was identified between the Se levels in rice grains and those in the soil ($r = 0.316$), as well as between rice grain Se levels and those in the roots ($r = 0.464$) and shoots ($r = 0.488$). Conversely, negative correlations were observed between soil Se, root Se, shoot Se, and grain Se with EC, with correlation coefficients of $r = -0.197$, $r = -0.163$, $r = -0.161$, and $r = -0.118$, respectively. The data indicates that the Se in the soil, roots, shoots, and grains had a weak correlation with SOM, with correlation coefficients of $r = 0.230$, $r = 0.281$, $r = 0.207$, and $r = 0.121$, respectively (Table S4).

In summary, noteworthy relationships were identified between the Cd level in rice grains and their presence in the soil ($r = 0.447$), roots ($r = 0.235$), and shoots ($r = 0.375$) (Fig. S4). The correlation between Cd levels in the roots and shoots and SOM appeared weaker, with correlation coefficients of $r = 0.196$ and $r = 0.097$, respectively. Furthermore, a negative correlation was observed between Cd in the roots and EC ($r = -0.159$). In the Punjab region, there were notable associations between the levels of Cd in rice grains, which were significantly linked to those in the soil ($r = 0.486$), roots ($r = 0.223$), and shoots ($r = 0.369$). Additionally, there was a positive correlation between SOM and EC, with a correlation coefficient of $r = 0.195$.

These correlations suggest that the Se and Cd present in the soil and plant tissues significantly influence the accumulation of Se and Cd in rice grains. The soil, root, and shoot Se and Cd levels had the highest correlations with the rice grain Se and Cd levels. The soil pH also increased the Se level and decreased the Cd level in the soil system to some extent, which eventually increased the accumulation of Se and decreased the Cd accumulation in the rice plant system. The study area's negative correlation between EC, Cd, and Se levels in soil and plant tissues highlights the complex interactions between soil properties, plant physiology, and environmental factors influencing nutrient uptake and availability. The EC often indicates a higher concentration of ions in the soil solution (*e.g.*, potassium, calcium, magnesium). These ions can significantly compete with Cd and Se for uptake by plant roots (de Livera et al., 2011; Kikkert and Berkelaar, 2013). In contrast, the SOM level slightly increased the Se and Cd levels in paddy soil, eventually increasing Se and Cd accumulation in plant tissues. There were no significant correlations between Cd levels in rice grains and soil properties in the Sindh study area.

Our findings suggest an inverse correlation between the Se and Cd concentrations in rice grains. A similar pattern was observed for Se; the Se concentrations in the paddy shoot and root tissues were inversely correlated with the Cd concentrations in the plant shoot and root tissue samples (Fig. S5). The inverse correlation between Se and Cd in paddy grains was further characterized as follows: Se deficiency ($>Se 0.04$ and $>Cd 0.2$ mg/kg), no risk ($>Se 0.04$ to 0.3 and $<Cd 0.2$ mg/kg), high Cd risk ($<Se 0.04$ and $>Cd 0.2$ mg/kg), Se risk ($>Se 0.3$ and $<Cd 0.2$ mg/kg), Cd risk ($>Se 0.04$ to 0.3 and $>Cd 0.2$ mg/kg), and Se-Cd co-exposure risk ($>Se 0.3$ and $>Cd 0.2$ mg/kg) (Fig. 3). 42.6 % of grain samples with moderate Se and low Cd levels ($r = 0.07$, $p \leq 0.01$) signified no possible consumption risk for humans. The remaining categories indicate differing degrees of risk. The low Se and low Cd 31 % ($r = 0.08$, $p \leq 0.01$) and 20 % of samples ($r = 0.13$, $p \leq 0.01$) with high Se and low Cd grain samples signify Se deficiency ($<Se 0.04$ mg/kg) and Se risk ($>Se 0.3$ mg/kg) for humans in the study area. Elevated Se levels and low Cd levels in the rice samples indicated the potential for severe Se exposure hazard due to abundant rice intake. The intake of large amounts of Se through rice causes selenosis; however, in the current study, the occurrence of selenosis due to rice consumption seems unlikely because selenosis primarily occurs following the consumption of >0.8 mg/day

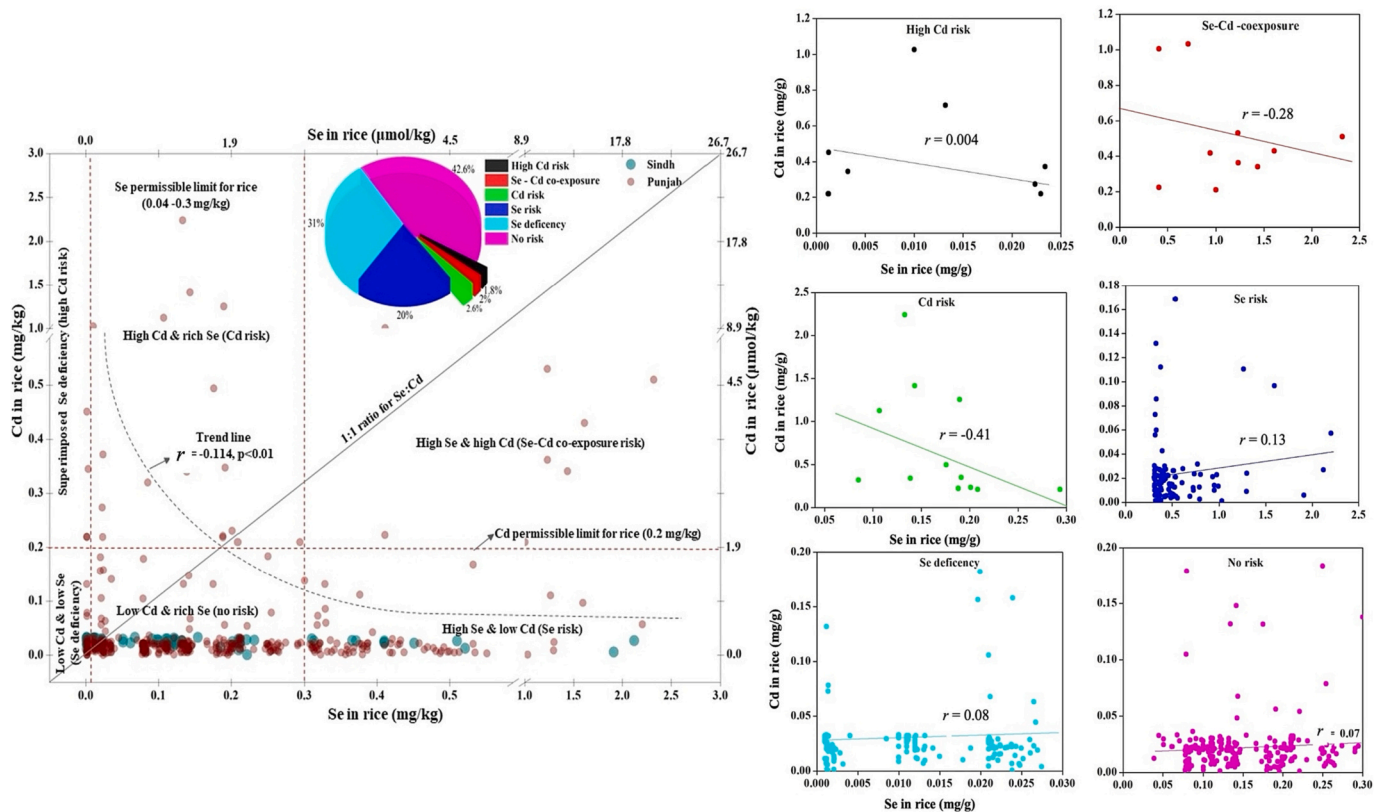


Fig. 3. Correlation between Se and Cd concentrations in rice grains and risk classification: low Se and low Cd levels (Se deficiency), low Se and high Cd levels (Se deficiency and high Cd risk), moderate Se and high Cd levels (Cd risk), moderate Se and low Cd levels (no risk), high Se and low Cd levels (Se risk), and high Se and high Cd levels (Se and Cd co-exposure risk).

(Dinh et al., 2018). As per WHO guidelines, the chronic consumption of high amounts of Se through rice must be averted to control Se intake, which should not exceed 0.4 mg/day (Organization, 1996).

However, the Cd risk of grain samples 2.6 % ($R = -0.41$, $p \leq 0.01$) with high Cd and rich Se, high Cd risk 1.8 % ($R = 0.004$, $p \leq 0.01$) with superimposed Se deficiency high Cd, and Se-Cd co-exposure risk 2 % ($R = -0.28$, $p \leq 0.01$) with high Se and high Cd indicated the potential for Cd exposure hazard due to abundant rice intake. The Se: Cd molar ratios of almost all rice samples showed moderate levels of Se and high levels of Cd > 1 , indicating the potentially limited ability of Se to antagonize Cd and the Cd risk to residents who consume rice in this category. The lack of combined risk assessments for Se-Cd co-exposure in humans and animals complicates the ability to draw definitive conclusions about grain samples containing elevated levels of Se and Cd (Chang et al., 2022; Zwolak, 2020). The toxic effects of Cd on humans or animals might be averted to some extent through the consumption of acceptable levels of Se (J. Chen et al., 2020; Guo et al., 2021). For people living in areas with Se- and Cd-polluted soils, it is highly recommended to significantly restrict rice consumption with large amounts of Cd, particularly rice, and a molar ratio of Se to Cd > 1 to inhibit unnecessary Cd accumulation in the body. The rice samples with low Se and Cd concentrations from Sindh and Punjab Provinces strongly indicate Se inadequacy. In this study, the number of rice samples in the Se deficiency category was highest, followed by the numbers of samples in the no-risk, Se risk, Cd risk, Se-Cd co-exposure risk, and high Cd risk categories.

4.3. Se and Cd translocation and bioaccumulation in paddy plant systems

In plant systems, metal TF and BAFs define the ratio of the metal levels in plant systems to that in soil systems, indicating metal translocation and accumulation ability in plant systems (Wang et al., 2014).

The current study applied TF and BAFs to explain the Se and Cd translocation and bioaccumulation patterns in paddy plant tissues (Fig. 4 and Table S5). The results indicate that paddy roots have the highest concentrations of Se and Cd across the study areas. This relatively large accumulation of Se and Cd in paddy roots is mainly attributed to the specific role of roots in translocating, adsorbing, and retaining Se and Cd from soil to plant roots and from plant roots to plant shoots and grains (Khanam et al., 2020). Therefore, paddy roots form the core route by which plants can take up and translocate Se and Cd from paddy soil to paddy tissues such as shoots and grains.

In contrast, uptake by paddy leaves can be ignored, especially in regions far from areas with relatively high levels of atmospheric Se and Cd deposition. The BAFs of Se were highest throughout the study area in the paddy roots, followed by plant shoots and grains, while the TF of Se remained highest in the plant shoots, followed by grains. Interestingly, the BAFs of Cd were highest in the paddy roots, followed by the grains and shoots. The BAFs of 24.2 %, 6.8 %, and 2.6 % of the samples exceeded 1, suggesting a higher accumulation of Cd in plant tissues. The TF of Cd was highest in the grains, followed by the shoots, with TFs of 7.8 % and 10.4 % of the samples, respectively, exceeding 1, indicating higher translocation of Cd from the roots to the aerial tissues, i.e., grains and shoots. In paddy tissues, Se and its species, i.e., selenite (SeO_3^{2-} or Se^{4+}) and selenate (SeO_4^{2-} or Se^{6+}), are mainly transported by phosphate and sulfate transporters. In roots, Se usually accumulates in the root cell vacuoles and cavities in the tonoplast; Se moves within plant cells via transporters and is translocated to other plant tissues, such as leaves, shoots, and grains (Ali et al., 2020).

Translocation of Cd^{2+} from paddy soil solutions to plant tissues is facilitated by the ZIP (OsIRT1 and OsIRT2), OsNramp5, and OsHMA3 transporters. ZIP transporters play a critical role in paddy plants, indicating Cd^{2+} ion influx activity and receptivity of paddy plants to Cd^{2+} ions, which are significantly related to the intensity of ZIP expression.

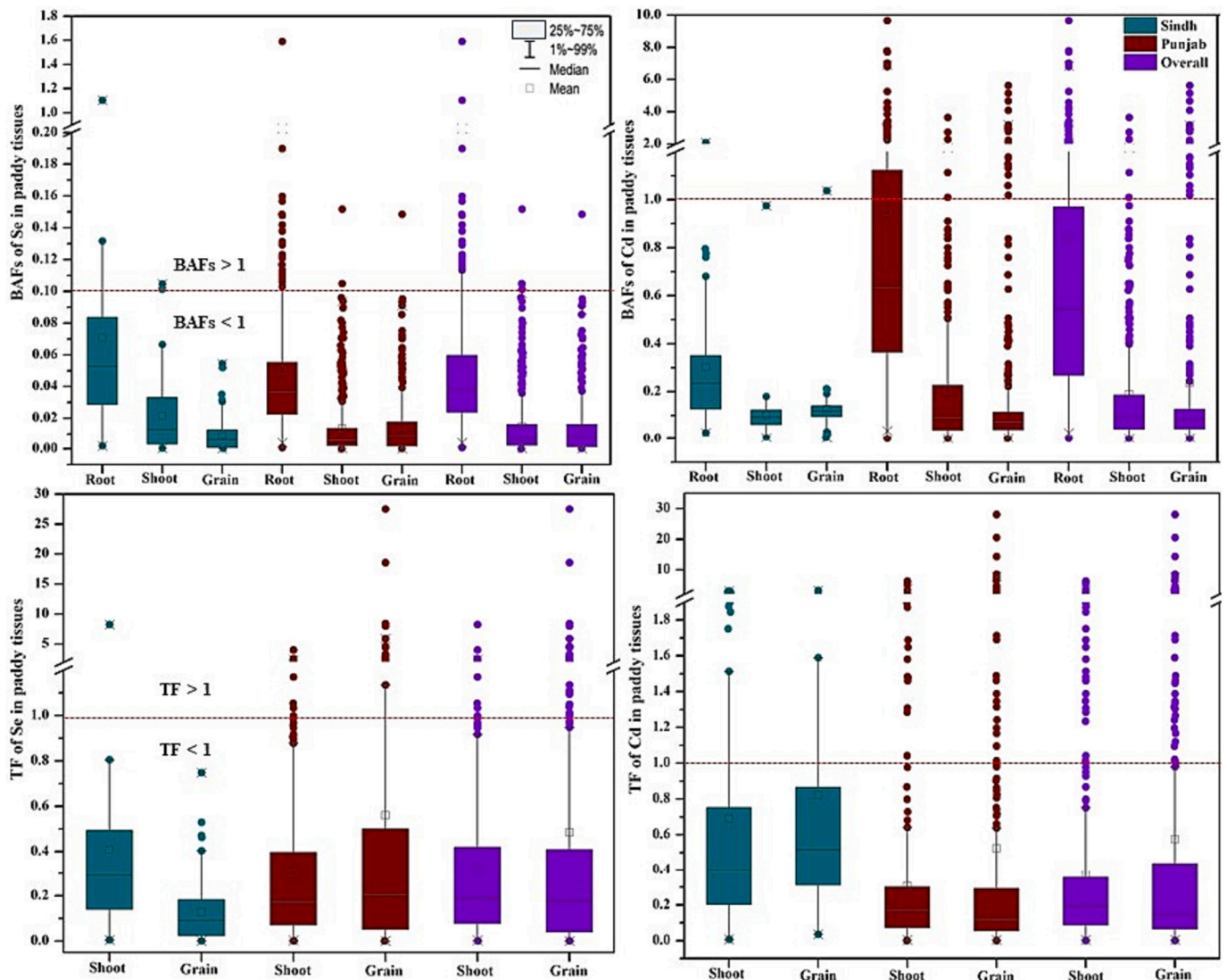


Fig. 4. Patterns of the bioaccumulation factor (BAFs) and translocation factor (TF) related to the concentrations of Se and Cd in rice plant tissues.

Thus, these ZIP transporters are crucial for Cd^{2+} ion uptake via root tissues in paddy fields (Ali et al., 2020; Uraguchi and Fujiwara, 2012). The OsNramp5 transporter, mainly located in the outer layer and endodermis of root tissue, can also capture Cd^{2+} ions from the soil solution (Sasaki et al., 2012). When root tissues take up Cd^{2+} ions, the biochemical actions that could detoxify Cd in grains are stimulated (Sterckeman and Thomine, 2020). The OshMA3 transporters, generally located in the vacuoles of paddy root tissues, also facilitate the transportation of Cd^{2+} ions into vacuoles. These ions can further decrease Cd from paddy root tissues through free sulfhydryl groups to shoot tissues, eventually causing Cd accumulation in roots (H. Li et al., 2017; Sasaki et al., 2014). Laboratory-based studies suggested the overexpression of OshMA3 and, ultimately, decreased Cd accumulation in paddy grains (Lu et al., 2019; Sasaki et al., 2014). Likewise, stable isotope confirmation of Cd has proven that mainly lighter Cd isotopes are retained in paddy root tissues, while only heavier Cd isotopes can translocate to the aerial tissues of paddy plants (Wiggenhauser et al., 2021; Yan et al., 2021; Zhang et al., 2021).

In the current study, the Cd concentration, TF, and BAFs of paddy grains were slightly higher than those of paddy shoots (roots > grains > shoots); this contrasted with the results of other studies conducted in a neighboring country (China) in which the order was roots > shoots > grains. The findings indicate that the elevated levels of Se species, total

reductive Se, and strong inverse Se interaction on Cd play a pivotal role in inhibiting Cd uptake by rice tissues (Farooq and Zhu, 2019; Li et al., 2020; Yan et al., 2021). The results of this study suggested the strong ability for xylem translocation and bioaccumulation of Cd from the roots to the grains of paddy in all the study areas.

4.4. Effects and underlying mechanisms of Se on the translocation and accumulation of Cd in grains

Paddy soil is a significant source of Se in plant tissues, whereas tissues such as leaves can take up minimal concentrations of atmospheric Se (Chang et al., 2019; Zhou et al., 2017). Likewise, the overall positive correlations between the Se levels in grains and paddy soil ($R^2 = 0.447$), plant roots ($R^2 = 0.564$), and plant shoots ($R^2 = 0.584$) and between the Cd concentrations in the rice grains and paddy soil ($R^2 = 0.477$), plant roots ($R^2 = 0.235$), and plant shoots ($R^2 = 0.375$) at a significance level of $p = 0.05$ (Figs. S3 and S4). The paddy roots and shoots significantly contributed to the Se and Cd levels in the rice grains, and both Se and Cd were taken up mainly from the paddy soil. Overall, there was a positive correlation between levels in the paddy soil and plant tissues, roots, shoots, and grains; these correlations are consistent with general laboratory-based findings (Li et al., 2020; Liu et al., 2019) and studies conducted in the southwestern karst region of the northwestern Baiyin

district, China (Liu et al., 2019; Wen et al., 2020). Consequently, the total increase in paddy soil Cd resulted in increased Cd concentrations in the rice plant roots, shoots, and grains. Interestingly, the samples collected from the study areas of Sindh Province did not show a positive correlation between the Cd level in the paddy soil and the plant tissues, roots, shoots, and grains. Therefore, it is speculated that in the Sindh study area, the Cd translocation from the paddy soils to the plant tissues was inhibited and altered; the nonpositive correlations supported this speculation among the Cd concentrations in the paddy soils and the plant's tissues, roots, shoots, and grains ($p = 0.05$).

Selenium is an excellent mediator of Cd-polluted soils. Using exogenous Se can lower the concentration of Cd released in the soil solution. It reduces Cd's bioavailability, uptake, and accumulation capacity and limits Cd's translocation from roots to aboveground plant tissues (Liu et al., 2020). Following exogenous Se application to a soil system, root secretions, and soil microbes shift the Se valence state; that is, the reduction in SeO_4^{2-} or SeO_3^{2-} ions to elemental selenium (Se^0) or selenide (Se^{2-}) and the formation of selenium-cadmium (Se-Cd) or cadmium-selenite (CdSeO_3) complexes are stimulated, thus lessening Cd toxicity (Qi et al., 2021). A study reported a comparable ending about the impacts of Se on the Cd uptake and accumulation in plant tissues and Cd bioavailability in a paddy soil system under anaerobic flooding conditions (Huang et al., 2018). This study applied 1 mg/kg sodium selenite (Na_2SeO_3) to Cd-polluted paddy soil. The results showed that Na_2SeO_3 not only substantially inhibited Cd pollution by reducing the bioavailable Cd by 12 % but also converted the available Cd into less available carbonate (CO_3^{2-}) or iron-manganese oxide ($\text{Fe}_2\text{Mn}_2\text{O}_4$) fractions (Huang et al., 2018). Similar results by adding SeO_3^{2-} or SeO_4^{2-} to paddy plant hydroponic experiments also showed SeO_3^{2-} had a comparatively high Cd reduction rate, reaching 41.4 % (Wan et al., 2016). In the current study, the range of Se concentrations in the paddy soil was significantly higher than the amount of Se added to the soil in the abovementioned study.

Likewise, the atypical noncorrelation of the Cd concentrations in paddy soils and tissues may signify that the uptake and Cd translocation from soil to roots was generally inhibited. It is assumed that a significant interaction may occur between Se and Cd in the soils of alluvial-flooded paddy fields across the study areas. Both extensive levels of reductive Se species and total Se in the paddy soil system might play a protuberant role in reducing Cd uptake in the rice system (Fig. 5). Several studies have confirmed that under flooded conditions, primarily in soil water, the Se transformation of species from a high valance state to a low valance state, i.e., $\text{SeO}_4^{2-} \rightarrow \text{SeO}_3^{2-} \rightarrow \text{Se}^0 \rightarrow \text{Se}^{2-}$ occurs, and extensive formation of CdSeO_3 or Cd-Se complexes are caused by a decrease in Cd toxicity (Huang et al., 2018; Riaz et al., 2021; D. Wang et al., 2019a). A

Se:Cd molar ratio of >0.7 (Shanker et al., 1996) in paddy soils can efficiently inhibit Cd accumulation in plant systems (Zhang et al., 2019). In the current study, the paddy soil's average Se:Cd molar ratio was >12.5 , indicating an abundance of reductive Se that can interact with Cd in paddy soil.

In the soil system, bacterial biogeochemical processes could also stimulate Cd-Se complex formation; for example, *Pseudomonas stutzeri* TSS4 can effectively initiate the formation of Se^0 and Cd-Se by catalyzing SeO_3^{2-} reduction (D. Wang et al., 2019a). This Cd-Se complex has relatively low bioavailability for plants in paddy soil systems. It cannot be efficiently translocated to underground plant tissues (roots), ultimately inhibiting Cd accumulation in aboveground paddy tissues (rice plant shoots, leaves, and grains) (Navarro et al., 2012). Selenium can also efficiently inhibit Cd mobilization among tissues through direct or indirect interactions with Cd in the plant system. The probable mechanisms of Se and Cd translocation and interaction in the alluvial flooded paddy soil-rice systems assessed in this study are shown in Fig. 5. Paddy roots are considered the main zone for the binding of Cd to Se (Huang et al., 2020). In the current study, a negative relationship was observed between the Se concentration in root tissues and the TF of Cd ($\text{Cd}_{\text{rice grain}}/\text{Cd}_{\text{root tissues}}$) $r = -0.16$, $p < 0.01$ (Fig. 6), signifying that the increased root Se concentrations mainly inhibited the translocation of Cd from roots to grains. Selenite is a primary and common Se species in paddy rice roots that can take up Se from paddy soils (H.-F. Li et al., 2010). After being absorbed by paddy roots, SeO_3^{2-} can instantly transform into the organic form $\text{Se}(\text{Se}^{2-})$ (X. Chen et al., 2020). In paddy systems, elevated Se concentrations contribute substantially to seleno-proteins or selenocysteine (Se-Cys), which are rich in selenols ($-\text{SeH}$), i.e., plasma glutathione peroxidase (GSH-Px) (Ali et al., 2021; Rayman, 2012). Furthermore, these elevated Se concentrations can promote the formation of GSH and phytochelatins (PCs) rich in sulfhydryl ($-\text{SH}$) functional groups, which can chelate more Cd^{2+} ions (Cao et al., 2018; Pons et al., 2021).

Moreover, these $-\text{SH}$ functional groups can be substituted with $-\text{SeH}$ groups because of the elevated chemical activity of $-\text{SeH}$ (Chang et al., 2020). PCs with $-\text{SeH}$ groups can substantially chelate Cd^{2+} ions and create Cd-Se complexes, which are transported to paddy root tissue vacuoles and accumulate, thus decreasing Cd mobility from underground root tissues to aboveground tissues (shoots, leaves, and rice grains) (Ismael et al., 2019). The inhibitory effects of elevated Se on Cd movement lead to the accumulation of specific levels of Cd in paddy root tissues and a decrease in the concentrations of Cd in rice grains, as observed in the current study. Thus, it is proposed that in the flooded alluvial paddy soil-rice system, the probable production of passive Se-Cd complexes and elevated rate of formation of Se-Cd complexes that are

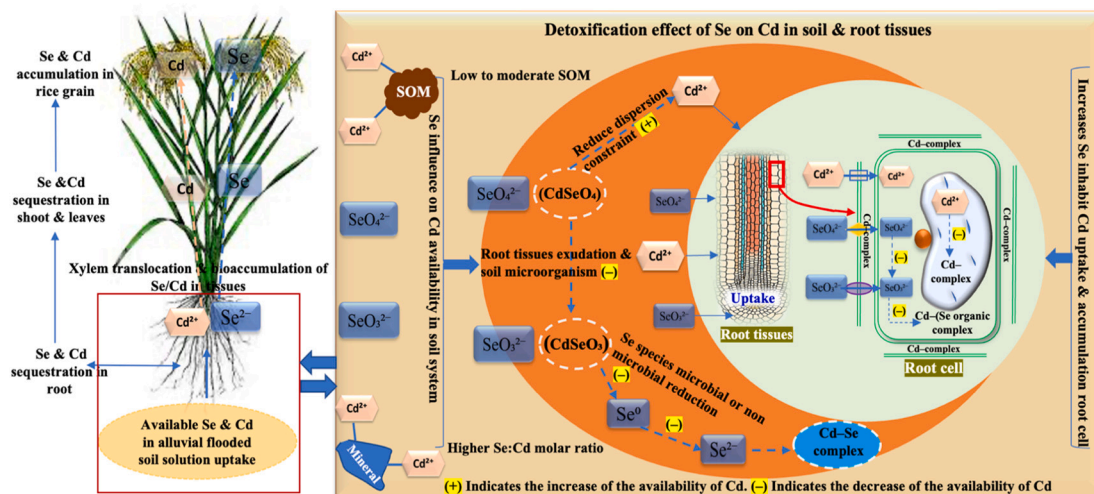


Fig. 5. The interaction between Se and Cd and the probable underlying mechanisms driving their interactions in the alluvial flooded paddy soil-rice system.

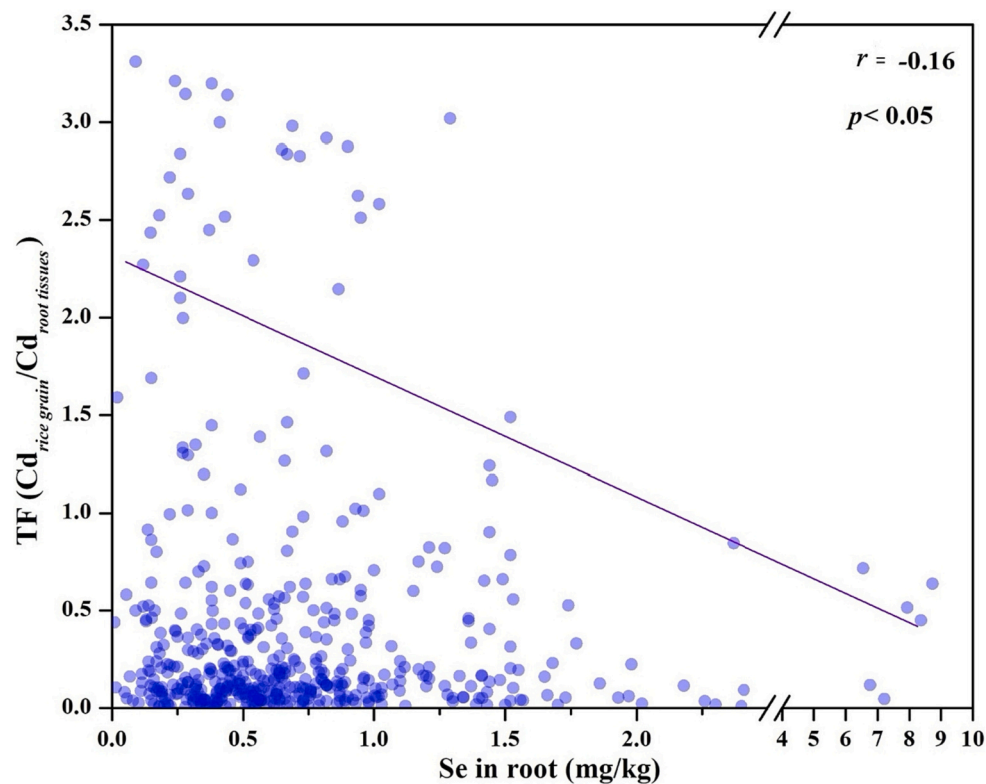


Fig. 6. Translocation factor (TF) of ($Cd_{\text{rice grain}}/Cd_{\text{root tissues}}$) compared with the effect of the root Se concentration.

stimulated by the increased Se concentrations in the roots are responsible for the decreased in the Cd concentration in rice grains.

5. Conclusion

In this study, naturally flooded alluvial regions with high Se levels and elevated Se:Cd molar ratios are conducive to interactions between Se and Cd concentrations in soil and rice ecosystems in Pakistan. The negative correlations between Se and Cd levels in soil and various plant tissues (rice grains, shoots, and roots) and the TF of Cd in grains and Se in roots confirmed that the elevated Se levels in the roots inhibited the transfer of Cd from roots to grains. We also observed that the Se deficiency category had the largest quantity of rice samples, with the following categories in descending order of sample numbers: no-risk, Se risk, Cd risk, Se-Cd co-exposure risk, and high Cd risk. Notably, our findings indicate that grains with elevated Cd levels and either moderate or low Se levels present the most significant food safety hazard. The present study provided us with a valuable opportunity to thoroughly contemplate the development of a scientific approach for evaluating human risks and the potential dangers associated with paddy soils and rice, specifically in regions characterized by low Se and low Cd concentrations, as well as those with moderate Se and high Cd concentrations.

CRedit authorship contribution statement

Waqar Ali: Conceptualization, Data curation, Methodology, Software, Writing – original draft. **Kang Mao:** Conceptualization, Methodology, Writing – review & editing. **Muhammad Shafeeque:** Software. **Muhammad Wajahat Aslam:** Writing – review & editing. **Wei Li:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no competing financial interests.

Data availability

No data was used for the research described in the article.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31700401 and U20A20326), the Chongqing Technology Innovation Center of Environmental Protection and Equipment (CQHKYCZCX2023), China Postdoctoral Fellowship (2023H0367), the Youth Cross Team Project of CAS (JCTD-2021-17), and the Youth Innovation Promotion Association CAS (2023415). We appreciate the anonymous reviewers and the editor for enhancing the manuscript's quality with their constructive comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.168896>.

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