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Key Factors Controlling Cadmium and Lead Contents in Rice Grains of Plants Grown in Soil with Different Cadmium Levels from an Area with Typical Karst Geology

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Abstract: Cadmium (Cd) is a naturally occurring element often associated with lead (Pb) in the Earth's crust, particularly in karst regions, posing significant safety hazards for locally grown rice. Identifying the key factors controlling Cd and Pb content in local rice is essential under the natural soil condition, as this will provide a crucial theoretical foundation for implementing security intervention measures within the local rice-growing industry. This study collected three types of paddy field soils with varying Cd concentrations from karst areas for pot experiments. The rice varieties tested included a low-Cd-accumulating variety, a high-Cd-accumulating variety, and a locally cultivated variety. Soil physicochemical properties and plant physiological indices were monitored throughout the rice growth stages. These data were used to construct a segmented regression model of Cd and Pb levels in rice grains based on the plant's metabolic pathways and the structure of polynomial regression equations. Stepwise regression identified the key factors controlling Cd and Pb accumulation in rice grains. In conclusion, the key factors controlling Cd and Pb levels in rice grains should be classified into two categories: (i) factors influencing accumulation in roots and (ii) factors regulating transport from roots to grains. The aboveground translocation abilities for Cd, Pb, zinc (Zn), iron (Fe), manganese (Mn), calcium (Ca), and magnesium (Mg) in soil among the three rice varieties showed no significant interspecific differences under identical soil conditions. Soil Mg uptake by rice roots may represent a key mechanism for inhibiting soil Cd uptake by rice roots. In karst areas with high background soil Cd, increased soil organic matter (SOM) levels enhance Pb bioavailability. Additionally, the rice YXY may possess a potential for low Cd accumulation.

Keywords: combined cadmium–lead pollution; soil–rice system; polynomial regression; mediating effects; interaction effects

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1. Introduction

Carbonate rocks are extensively distributed worldwide. In Southwest China, the karst region centered around Guizhou is one of the world's major karst areas [1]. This southwestern karst region of China is a principal site for low-temperature mineralization [2], characterized by a diverse and abundant array of mineral resources, with evident heavy metal enrichment in the soil. In the karst regions of Southern Guizhou and Northern Guangxi, soil Cd concentrations exhibit elevated background values [3,4]. This heightened presence of Cd arises from the combined processes of parent rock weathering into the soil and the mineralization of heavy metals within the region [5,6]. Additional pollution sources include fertilization with impure mineral phosphate fertilizer [7,8], irrigation with contaminated water, and surface runoff from mining tailings [9,10]. Soil heavy metal pollution often involves the simultaneous introduction of multiple elements into the soil–plant system, significantly influencing crop yield and the redistribution of elements within the crop [11]. Macnicol et al. [12] introduced the concept of combined toxic effects. Cd, dispersed in nature, is often enriched in associated forms in metallic sulfide deposits, bauxite, and coal beds, leading to frequent soil contamination by complexes, which increases the risk for rice farming. Cd, which is more mobile than Pb, is typically more susceptible to competition [13,14].

Rice (*Oryza sativa* L.), a locally significant crop, faces safety hazards during cultivation. Compared to other grains, rice tends to accumulate higher levels of Cd [15–17], with notable variances among different rice varieties [18]. Indica rice and strains containing Indica genes typically accumulate more Cd than Japonica varieties [19,20]. In the Qian-Gui region, where Indica rice predominates, food safety faces substantial challenges. In 1972, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations identified Cd as a food contaminant posing severe health risks. In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended a tolerable monthly intake of 25 μg of Cd for humans [21]. In China, Wang et al. (2018), using dietary data from Song's survey on Cd contents in food across 31 provinces and the China Nutrition and Health Survey, extrapolated the average monthly Cd intake per unit body mass for Chinese adults to be 15.3 μg (61.2% of the JECFA recommended value). They postulated that this recommended value would be exceeded after 35 years [22,23]. The International Agency for Research on Cancer (IARC) has classified Cd as a class I carcinogen [24]. Zhao concluded that the high rate of rice consumption in China necessitates the implementation of a stringent limit on Cd in Chinese rice. The maximum allowable limit of Cd in Chinese rice is set at 0.2 mg/kg, which is half of the FAO/WHO recommended value of 0.4 mg/kg [25].

Xia et al. [26] uncovered discrepancies among the Chinese Agricultural Land Standard (Soil Standard) [27], Cd limit values in brown rice as stipulated in the Chinese Food Standard [28], and soil Cd screening values in Soil Standard. Similarly, Wang et al. [29] highlighted issues with the Soil Standard, emphasizing the variability of factors influencing Cd accumulation in rice across different environments and the importance of rectifying such discrepancies. Their findings indicated a weak correlation between Cd levels in crops and total soil Cd. Due to the diverse and complex distribution patterns of heavy metals across distinct chemical species or solid phases, measurements of total heavy metal content cannot accurately estimate their bioavailability and toxicity in soils [30]. Correspondingly, multivariate linear regression models using soil physicochemical characteristics and total heavy metal concentrations to predict Cd levels in rice grains generally yield unsatisfactory results [31], as heavy metal uptake by crops is influenced by numerous factors [32,33]. Compared to total soil Cd, biologically available Cd serves as a better indicator for assessing the risk of Cd accumulation in rice grains. However, accurate predictive methods for this purpose are currently lacking [34].

Cd, a non-essential element for plants, not only retards growth but also induces leaf yellowing and even mortality. Roots are the primary organ responsible for Cd absorption and accumulation in rice plants, directly influencing the aboveground translocation of Cd [35]. During vegetative growth, rice absorbs various elements from the soil for the normal development of roots, stems, and leaves and to accumulate essential nutrients for the reproductive stage. Toxic heavy metals in the soil enter rice roots through ion channels for other nutrient elements [36], translocate to aboveground parts during growth and metabolism, and ultimately accumulate in the grains. Transporter proteins essential for Cd uptake in rice roots also aid in absorbing necessary nutrients, including Fe, Mn, and Zn [37]. Throughout vegetative growth, most Cd absorbed by rice accumulates in the leaves [38]. Cd accumulated in leaves and stems before flowering significantly contributes to grain Cd [39]. During reproductive growth, starting with rice blossoming, the growing grain becomes the metabolic center, and nutrients and Cd from leaves and stems transfer to the grains [40,41]. It has been proposed that the period from blooming to grain filling is optimal for reducing Cd transport from stems and leaves to grains, as 69.82% to 84.53%

of Cd is absorbed from the soil before the heading stage [42]. Studies indicate that roots store between 49% and 79% of the total Cd ions entering the plant, with approximately 24% being potentially mobile [43]. Transport from roots to shoots governs eventual Cd accumulation in grains [44,45]. Some studies suggest that the grain-filling stage could induce strategies to alter the chemical speciation and transport of Cd in rice, but studies linking rice physiological performance to controlling Cd retention and transport are still lacking [46].

Currently, research on reducing Cd and Pb content in rice grains primarily focuses on foliar absorption blockers and soil heavy metal passivation technology [47]. Additionally, work commonly involves developing slow-release passivation and adsorption technology for soil heavy metals using porous media materials as carriers. Inorganic metal-based deterrents, such as soluble compounds of Zn, Fe, Mn, Ca, Se, and silicon (Si), are commonly used [48–51]. Nevertheless, there is a paucity of research examining the factors contributing to the antagonistic effects of soil Cd and soil Cd-Pb uptake by rice cultivated in a natural process in regions with a high Cd geological background.

The experimental design of this study involved analyzing rice field soils with varying Cd contents in the karst regions of Guangxi and Guizhou. The high geological Cd background in these areas presents significant safety hazards for rice cultivation, particularly regarding Cd content in rice grains. Therefore, it is crucial to identify the key factors influencing Cd and Pb contents in rice through their natural regulatory mechanisms. This understanding could eventually inform soil testing and formula fertilization, with the ultimate goal of reducing the Cd content in rice grain, providing a theoretical basis for implementing safe and reliable interventions in local rice cultivation. The soil's physicochemical characteristics and rice plant physiological indicators were monitored throughout the rice growth process. The experimental data elucidated the physiological responses of soil Cd and Pb accumulation and transport in rice plants, as well as their interactions with essential nutrients, including Zn, Fe, Mn, Ca, and Mg. This study employs a segmented model constructed through multivariate regression analysis based on the metabolic pathways of heavy metals in rice plants. The model uses second-order polynomial regression equations to expand the information dimension of the original independent variables, overcoming limitations of previous linear or nonlinear regression models and greatly improving the explanatory power of the original independent factors. Ultimately, the theoretical model was validated by examining the chemical speciation of soil heavy metals (BCR), which served as a means of assessing its reliability.

2. Materials and Methods

2.1. Experimental Soil

Three soils used in the pot experiment were sourced from paddy fields in typical karst geological regions. Two soil types (XQ and WP) were obtained from Yundong Township, Duyun City, Guizhou Province, known for their high geological Cd background (Figure 1a). The XQ soil was near a Pb-Zn mining area and was additionally influenced by anthropogenic pollution (Figure 1c), while the WP site was free from human pollution (Figure 1d). As a control, RA soil was sourced from Rongan County, Liuzhou City, in the Guangxi Zhuang Autonomous Region (Figure 1b,e). During collection, 12 bags of paddy field soil, each weighing approximately 30–35 kg, were obtained at each location, totaling 36 bags. Collection sites were situated in farmland areas more than 20 m away from main roads [52]. Each bag represented a composite sample from multiple points, collected using a five-point sampling method, with a depth range of 10 to 40 cm. On-site, the bags were weighed, sealed, numbered, and transported to the laboratory for further processing. As illustrated in Figure 1f, XQ soil exhibited the highest Cd contents, followed by WP and RA. Table S1 provides the physicochemical characteristics of the soils used in the pot experiment.

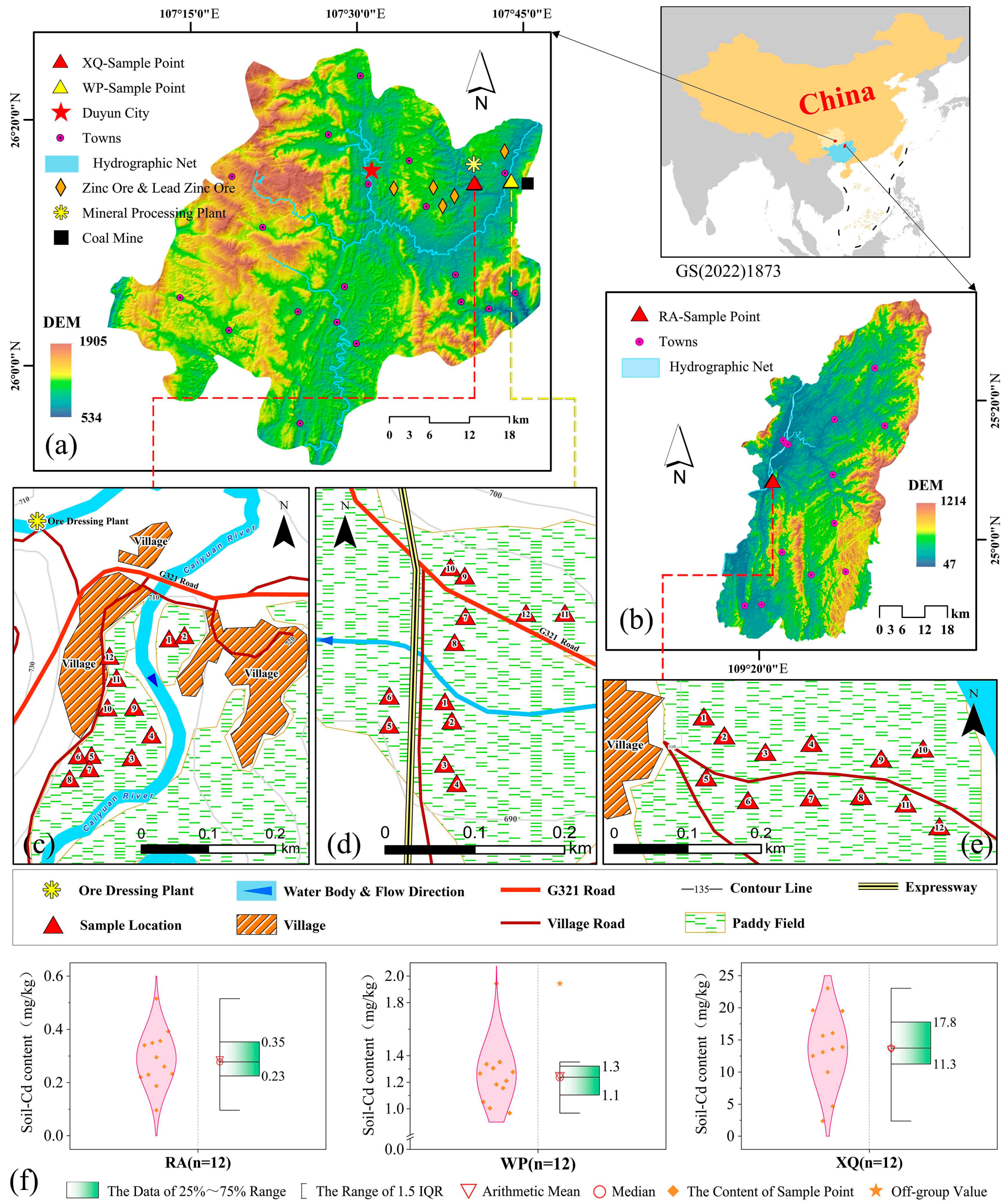

Figure 1. Collection situation of soil samples for testing. GS(2022)1873 refers to the base map review number from the Ministry of Natural Resources of China's standard map service system. The national, provincial, municipal (or state), and district boundaries depicted in (**a**–**c**) comply with the specifications of base map GS(2022)1873. Figure 1a shows the locations where potting soil samples were collected at XQ and WP in Duyun City, Qiannan Prefecture, Guizhou Province. (**b**) illustrates the sampling locations of RA potting soil in Rong'an County, Liuzhou City, Guangxi Zhuang Autonomous Region. (**c**–**e**) depict the distribution of actual sample points at XQ-Sample Point, WP-Sample Point, and RA-Sample Point, respectively. (**f**) presents the total Cd content in the potting soil sample points collected from the three locations, note the different y-axis ranges.

2.2. Experimental Rice Variety

Table 1 provides key information on the rice varieties (JLY, YXY, and ZZY) utilized in this investigation. All rice seeds were secured from local agricultural stations, and the rice seedlings had reached 30 days of age at the time of transplantation.

Table 1. Basic Information of Test Rice.

Type	Variety Name	Acronyms	Genealogy	Characterization	Parental Origin [a]
High-accumulation with Cd	Zhongzheyou No.1	ZZY	Indica three-line hybrid rice	Warm-season crop	Zhonghang A (♀), Hanghui 570 (♂)
Low-accumulation with Cd	Jingliangyou Huazhan	JLY	Indica two-line hybrid rice	Warm-season crop	Jing 4155S (♀), Huazhan (♂)
Local variety	Yexiangyou Simiao	YXY	Indica three-line hybrid rice	Warm-season crop	Yexiang A (♀), R Simiao (♂)

[a] ♀ It signifies the maternal origin of genes. ♂ It signifies the paternal origin of genes.

2.3. Experimental Design

Table S2 delineates the grouping arrangements for the pot experiments, while Figure 2 depicts the cultivation and sampling procedures, which are divided into five monitoring phases (phase 1 to phase 5) for the incubation process. Soil samples were gathered at various phases: before irrigation (phase 1); before transplanting (phase 2); at the booting stage (phase 3); at the full-heading stage (phase 4); and the full-ripe stage (phase 5). Additionally, each soil sample must weigh at least 1 kg per bag (wet weight).

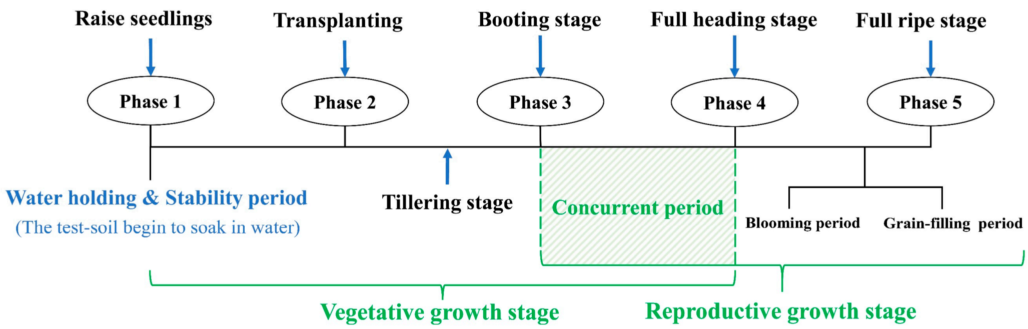

Figure 2. The Process of Rice Growth in a Pot Experiment.

Soil redox potential (E_h) was conducted before transplanting and at each phase. Soil samples were obtained at each phase and subjected to analysis to determine pH, cation exchange capacity (CEC), SOM contents, as well as the heavy metal contents of the total and each composition (BCR method: L1, L2, L3, R3) of chemical speciation, including Cd, Pb, Zn, Fe, Mn, Ca, and Mg. Whole rice plants were harvested at the full-heading stage and full-ripe stage, and their heavy metal contents (Cd, Pb, Zn, Fe, Mn, Ca, and Mg) were assessed in each part of rice, including the ears, leaves, stems, and roots.

2.4. Sample Preprocessing

The soil samples were initially air-dried indoors and then crushed with a wooden hammer to remove impurities. They were ground in an onyx mortar until they passed through a 0.85 mm nylon sieve. The sieved samples were thoroughly mixed and then divided into two portions using the quartering method. One portion was retained as a coarse-ground sample for determining soil pH and CEC, while the other portion was further ground until it passed through a 0.25 mm sieve. The fine-ground sample was then subdivided into two parts using the quartering method. One part was used to assess soil SOM, while the other underwent additional grinding to pass through a 0.15 mm sieve for heavy metal content analysis. These procedures were conducted in accordance with the guidelines set forth in the Technical Specification for Soil Environmental Monitoring (CN) [52]. For chemical speciation analysis (BCR) of soil metal elements, individual soil samples were prepared and passed through a 0.075 mm sieve.

The rice plant samples were rinsed with tap water to remove soil and surface impurities, followed by washing with deionized water. Samples intended to determine the dry weight of rice plants and heavy metal contents in various parts were individually packaged in bags, categorized by aboveground parts (grains/ears, leaves, stems) and below-ground parts (roots). The samples were initially subjected to a 20 min heating process at 105 °C, followed by a 24 h drying at 70 °C. Prior to weighing, the samples intended for determining the dry weight of various plant parts were oven-dried until reaching a constant weight [53]. Subsequently, the grains were weighed after undergoing the drying process, and the brown rice was retained through the dehulling procedure. Both the brown rice and the samples from the various plant parts that had been subjected to the drying process were pulverized and stored in polyethylene plastic bags.

2.5. Sample Detection and Analysis

The soil E_h was determined using the potential method [54], while soil pH was assessed employing the same technique [55]. SOM contents were determined using the potassium dichromate volumetric method–dilution heat method, with SOM values calculated from absorbance measurements obtained using a spectrophotometer [56]. Soil CEC was determined utilizing the hexamine cobalt trichloride solution–spectrophotometric method [57]. The total contents of heavy metals and nutrient elements in soil, including Cd, Pb, Zn, Fe, Mn, Ca, and Mg, were determined by analyzing soil extracts digested using the aqua regia–electric hot plate heating method [58]. The chemical speciation of soil metal elements was analyzed using the three-step extraction scheme of the BCR sequential extraction procedure (European Committee for Standardization). The first step targeted the mild acid-soluble fraction (L1), extracted using acetic acid. The second step focused on the reducible fraction (L2), extracted using a hydroxylamine hydrochloride solution. The third step addressed the oxidizable fraction (L3), extracted using hydrogen peroxide and ammonium acetate solution. The final remaining portion was the residual fraction (R3), determined by digestion with the aqua regia–electric hot plate heating method. This analysis included the contents of Cd, Pb, Zn, Fe, Mn, Ca, and Mg.

To determine the dry weight of each plant part, samples were dried to a constant weight and then weighed individually in bags at room temperature, with the weight of the dried bag subtracted to obtain the sample dry weight. Heavy metal contents (Cd, Pb, Zn, Fe, Mn, Ca, and Mg) in various parts of rice were determined by analyzing extracts obtained using the nitric acid–microwave heating method [59].

Analysis of the contents of heavy metals and nutrient elements in soil and rice samples initially employs inductively coupled plasma optical emission spectroscopy (ICP-OES). If elements not detectable by ICP-OES are encountered, further analysis is conducted using inductively coupled plasma mass spectrometry (ICP-MS).

2.6. Quality Control of Sample Detection

All reagents used in this experiment were of guaranteed reagent (GR) grade. Batch tests were conducted to analyze the SOM contents, CEC, soil total heavy metal contents, and rice heavy metal contents. Parallel tests were performed for each batch of samples, with 10% of the total sample quantity in each batch, ensuring a relative error within ±5% [60]. For quality control, certified reference materials were included in the analyses: limestone soil standard material GBW07404 (GSS-4) for soil total heavy metal contents analysis. Soil speciation composition was determined using the standard material GBW07444 (GSF-4) for each chemical speciation content of Cd and Pb in the soil. Hunan rice biological component analysis used standard material GBW10045a (GSB-23a) for heavy metal contents analysis in brown rice and biological component analysis standard material GBW10020 (GSB-11) for heavy metal contents analysis in other parts of rice. The recovery rates of standard materials fell within the range of 80% to 110%.

Samples were weighed using an OHAUS AR224CN balance (Shanghai Shenguang Instrument Co., Shanghai, China). A precision of 0.001 g was employed to determine the dry weight of rice plant parts, while 0.0001 g was used for other materials. Soil E_h was measured using an oxidation-reduction potential (ORP) meter (Model TR-901, Leici, Shanghai, China), and soil pH was measured using a pH meter (Model PHS-3C, Leici). Absorbance values of solutions were measured using a METASH V-5600 spectrophotometer (Shanghai Metash Instruments Co., Shanghai, China). The ICP analyses were conducted using a NexION300 ICP (PerkinElmer, Waltham, MA, USA), and the OES analyses were conducted using an Avio200 optical emission spectrometer (PerkinElmer).

2.7. Data Processing Methods

All data were entered and organized using Microsoft Office Excel 2019. Statistical analysis was performed using IBM SPSS Statistics 23. Graphs were generated using ArcGIS 10.7 and Origin Pro 2023.

Because the experimental data were discrete, a non-parametric correlation test (Spearman) was employed to analyze the indicators. When $|r| \geq 0.8$, the correlation is deemed high; within the range $0.5 \leq |r| < 0.8$, the correlation is considered moderate; and within the range $0.3 \leq |r| < 0.5$, the correlation is characterized as low. When $|r| < 0.3$, it indicates a very low linear correlation between the two variables, and they are considered unrelated [61].

The bioconcentration factor (BCF) is defined as the ratio of the concentration of an element in a specific plant organ to the concentration of that element in soil, as expressed by Equation (1). It signifies the ease with which rice roots absorb the element from the soil, serving as a crucial indicator of bioavailability [62,63]. The translocation factor (TF) is defined as the ratio of the concentration of an element in a specific plant organ to the concentration of the same element in another plant organ, as defined by Equation (2). TF is used to evaluate the transfer ability of the element between different organs of rice [62,63].

$$\boldsymbol{BCF} = \frac{C_i}{S} \quad (1)$$

$$\boldsymbol{TF} = \frac{C_i}{C_j} \quad (2)$$

In the equations, C_i and C_j represent the element contents in different parts of rice, where i denotes a specific aboveground part of rice; j represents the rice roots, and S represents the total contents of the element in the soil.

Equation (3) represents the fundamental form of the second-order polynomial regression model [64].

$$y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \beta_{11} x_{i1}^2 + \beta_{22} x_{i2}^2 + \beta_{12} x_{i1} x_{i2} + \varepsilon_i \quad (3)$$

In the equation, β_0 represents the constant term; $\beta_1 x_{i1}$ and $\beta_2 x_{i2}$ are linear terms representing the variables with a linear relationship with the dependent variable; $\beta_{11} x_{i1}^2$ and $\beta_{22} x_{i2}^2$ are quadratic terms representing the variables with a quadratic (nonlinear) relationship with the dependent variable; $\beta_{12} x_{i1} x_{i2}$ is the cross-product term representing variables with an interaction effect on the dependent variable, and ε_i is the error term.

The process by which rice absorbs Cd and Pb from the soil through its roots and then transfers them to the grains depends on the plant's metabolic mechanisms (Figure 3). Due to the intricate interplay between dependent and independent variables, incorporating diverse influences (linear, nonlinear, mediating, and interactive effects), polynomial regression emerges as a more efficacious method for accurately approximating the measured values. This approach allows for a more precise representation of the actual relationships among the data. Consequently, to augment the information dimension of the original independent variables and align them with the genuine biochemical characteristics of rice plants, it is suggested to first transform independent variables using the structural properties of polynomial regression equations (Table S3). To mitigate overfitting, a second-order polynomial was employed in constructing the model [64].

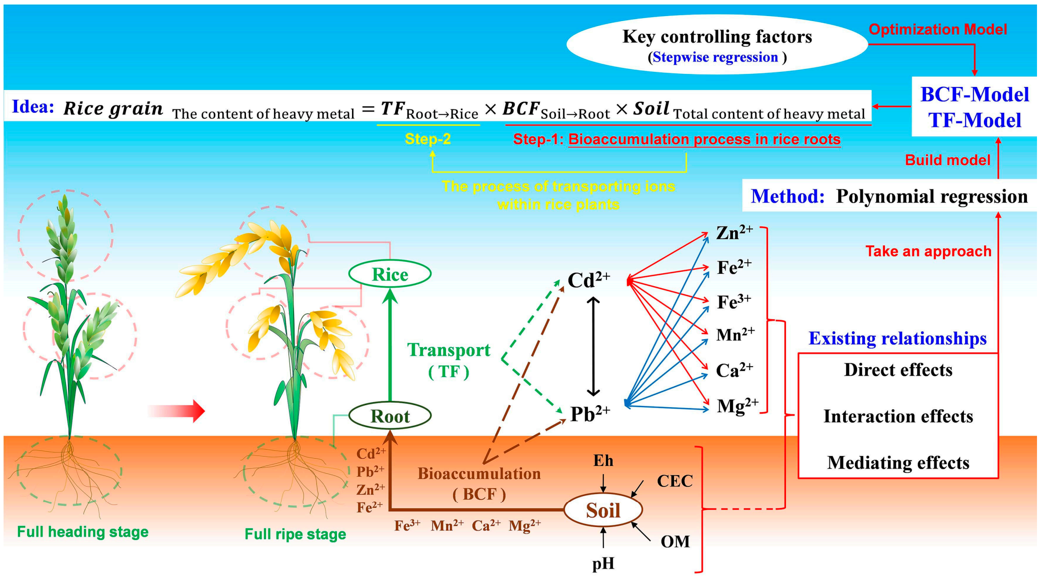

Figure 3. Metabolic Pathways and Interactions of Soil Heavy Metals in Rice Plants.

Analyses based on BCF and TF can offer a more intuitive understanding of the metabolic processes in rice involving heavy metals. The interpretation of rice TF, rice BCF, and total contents of soil heavy metal, as inferred from Equations (1) and (2) concerning heavy metal contents in rice grain, is represented by Equation (4).

$$\boldsymbol{Rice\ grain}_{\text{The contents of heavy metal}} = \boldsymbol{TF}_{\text{Root}\rightarrow\text{Rice}} \times \boldsymbol{BCF}_{\text{Soil}\rightarrow\text{Root}} \times \boldsymbol{Soil}_{\text{Total contents of heavy metal}} \quad (4)$$

where $\boldsymbol{Rice\ grain}_{\text{The contents of heavy metal}}$ represents the specific heavy metal contents in rice; $\boldsymbol{TF}_{\text{Root}\rightarrow\text{Rice}}$ represents the translocation factor indicating the transfer of the heavy metal from rice roots to grains; $\boldsymbol{BCF}_{\text{Soil}\rightarrow\text{Root}}$ represents the bioconcentration factor indicating the uptake of that heavy metal by rice roots from the soil, and $\boldsymbol{Soil}_{\text{Total contents of heavy metal}}$ refers to the total contents of that heavy metal in the soil.

Analyses based on the Bioconcentration Factor (BCF) and Translocation Factor (TF) can offer a more intuitive understanding of the metabolic processes in rice involving heavy metals. The interpretation of rice TF, rice BCF, and the total contents of soil heavy metals, as inferred from Equations (1) and (2) concerning heavy metal contents in rice grain, is represented by Equation (4).

Prior to constructing the model, correlation analysis and regression fitting were employed to establish the relationship between the dependent variable (y) and the independent variable (x) in rice in phase 4 and phase 5 (Table S4). Indicators with low correlation coefficients and those exhibiting polynomial fitting results deviating by less than 10% from linear fitting results were excluded from the models. This process aimed to ascertain the true linear and nonlinear mathematical relationships between y and x.

The optimal subsets for the TF and BCF models for each soil type were identified through stepwise regression (Table S5), and the key controlling factors for rice grain Cd and Pb levels were determined. To validate the results of the stepwise regression, the significance test method for multiple linear regression [61] was employed. The testing methods and validation sequence comprised the following: (i) assessing the linear relationship of the regression model (F-test, $P_F < 0.05$); (ii) evaluating the regression coefficients (t-test, $P_T < 0.05$); and (iii) diagnosing multicollinearity in the regression model using the Variance Inflation Factor (VIF). Models with VIF ≥ 10 for the independent variables were excluded; (iv) retaining the regression model with the minimum sum of squared residuals (SSE).

3. Results

3.1. Pot Experiment Results

Figure 4 illustrates the evaluation of soil Cd and Pb risk levels based on the risk screening value (referred to as the screening value) and the risk intervention value (referred to as the intervention value), as specified in the Soil Standard (CN) [27]. It also includes the permissible limits (referred to as the limit value) for Cd and Pb in brown rice, as outlined in the Food Standard (CN) [28]. These standards allowed us to classify the results into four distinct scenarios. Additionally, the Cd and Pb contents in three rice varieties cultivated in the same soil type were examined to determine the actual impact of Cd and Pb enrichment among different rice varieties.

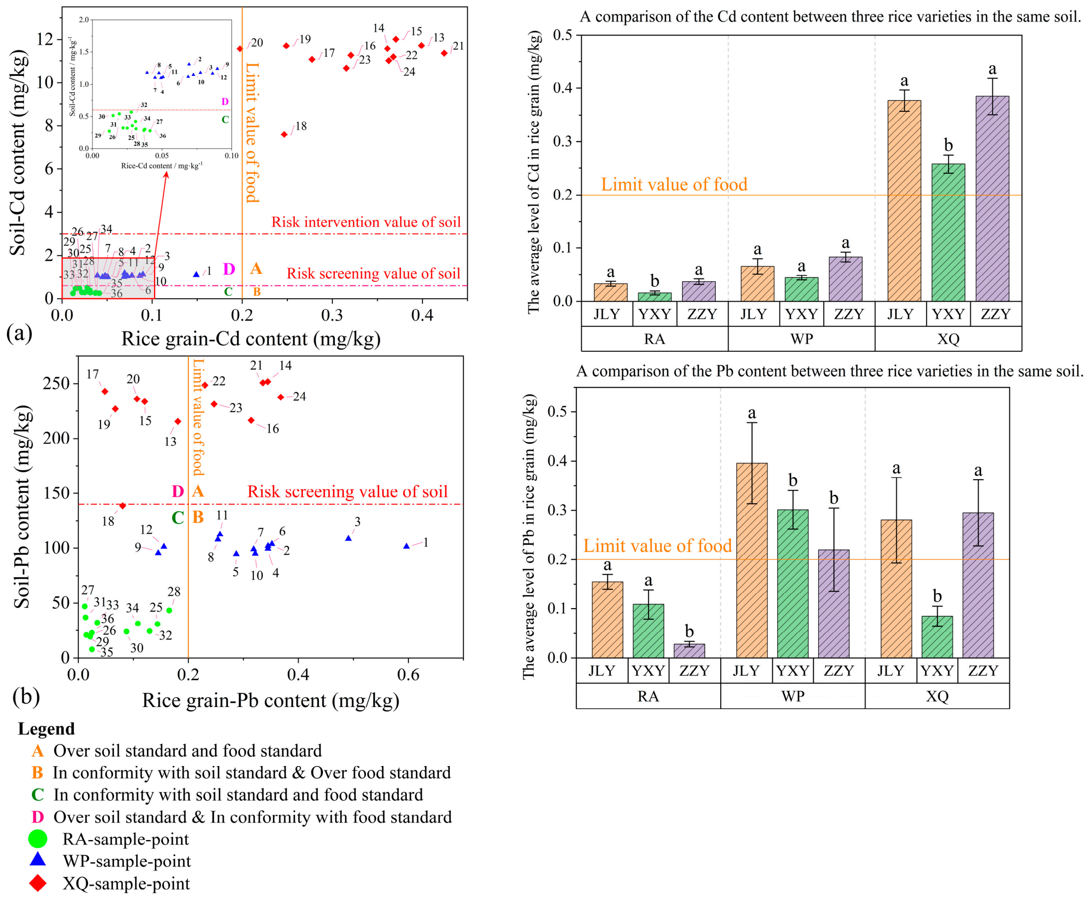

Figure 4. Analysis of Heavy Metal Content in the Pot Experiments. The abbreviations JLY, YXY, and ZZY refer to the names of the three rice varieties. (**a**) on the left depicts the correspondence between rice Cd content and soil Cd content in each group of soils, according to both food and soil standards. (**b**) on the left shows the correspondence between rice Pb content and soil Pb content in each group of soils, based on food and soil standards. The significance of differences between data was tested using Duncan's method ($p < 0.05$). The Cd and Pb contents in rice grains were compared among three varieties grown in the same soil. Differences are marked in the figures using lowercase English letters. When the markings between the different varieties do not share the same letter, a significant difference is indicated.

The RA soil served as the control in this experiment, with heavy metal levels in both rice grain and soil not exceeding the specified standards. The experimental results showed that rice YXY exhibited a significantly lower Cd content in the rice grain compared to the other two varieties. Conversely, rice ZZY demonstrated a significantly lower Pb content in the rice grain compared to the other two varieties.

In the WP soil, according to the screening value, there was a misjudgment regarding Cd contents: all soil Cd levels exceeded the soil limit, but grain Cd levels remained within the limit, resulting in a 100% misjudgment rate. Similarly, there was a misjudgment for Pb content: all soil Pb levels were within the limit, but almost all Pb levels in rice exceeded the limit, leading to an 83.3% misjudgment rate. Assessment based on the intervention value yielded a consistent misjudgment result for Pb levels compared to the screening value, and the Cd levels remained within the specified limit. The experimental results showed that Cd content in rice grain did not differ significantly among the three rice varieties; however, the Pb content in rice JLY was significantly higher than in the other two varieties.

In the XQ soil, according to the screening value, there was a misjudgment regarding Cd content: all soil Cd levels exceeded the soil limit, but only in one pot; the Cd levels in rice grain conformed to the specified limit, resulting in an 8.3% misjudgment rate. Additionally, there was a misjudgment for Pb content: soil Pb levels exceeded the soil limit, but half of the Pb levels in rice grain remained within the specified limit, leading to a 50% misjudgment rate. Evaluation based on the intervention value yielded a consistent misjudgment result for soil Cd and Pb levels compared to the screening value. This suggests that the soil in the XQ soil was contaminated with both Cd and Pb. The experimental results showed that Cd and Pb contents in the rice grains of YXY were significantly lower than in the other varieties.

The experimental findings revealed inaccuracies in assessing Cd and Pb contents in both soil and brown rice using these standards. Although cultivating low-Cd rice can significantly reduce grain Cd content, it may still exceed national food safety standards depending on the soil environment [65].

3.2. Rice Growth Status

In Figure 5a, a comparison of rice dry weight among the groups indicates that WP soil resulted in the highest biomass across the three rice varieties, while XQ soil exhibited the lowest, suggesting significant inhibition of rice plant growth in XQ soil. Furthermore, the grain yield of the three rice varieties was highest in WP soil, which is characterized by a high-Cd geological background. Previous studies have shown that low Cd stress can stimulate rice plant growth [66,67]. When comparing the dry weight of organs within the same rice variety across different soil types, no significant differences were observed in stem-leaf or root weight; however, notable discrepancies were found in the dry weight of the ears (grains) among the various soil types. This suggests that variations in rice dry matter under soil Cd stress primarily affect the ears. Figure 5c presents a comparative analysis of the biomass of the three rice species cultivated under identical soil conditions. The data indicate that rice YXY exhibited the highest biomass among the three species in all soil types. Additionally, the yields of rice YXY were significantly higher than those of the other two species. Rice YXY showed comparable yields to the low-Cd accumulating variety, rice JLY, in both geologically high-Cd background zones (XQ and WP). Conversely, rice ZZY demonstrated the poorest performance in both zones.

In Figure 5b, we compared the proportion of dry matter for each part of the same rice variety across different soil types. No significant differences were observed in the dry matter proportions of the ears or stem-leaf parts. However, the proportion of dry matter in the roots was notably higher in plants grown in XQ soil compared to those grown in the other soil types. This suggests a pronounced inhibitory effect on the aboveground growth of rice plants in XQ soil, resulting in a significantly higher proportion of dry matter allocated to the roots. Figure 5d presents a comparative analysis of the biomass percentage of the three rice varieties cultivated under identical soil types. The data indicate that the three rice varieties exhibited similar growth results, irrespective of the soil type.

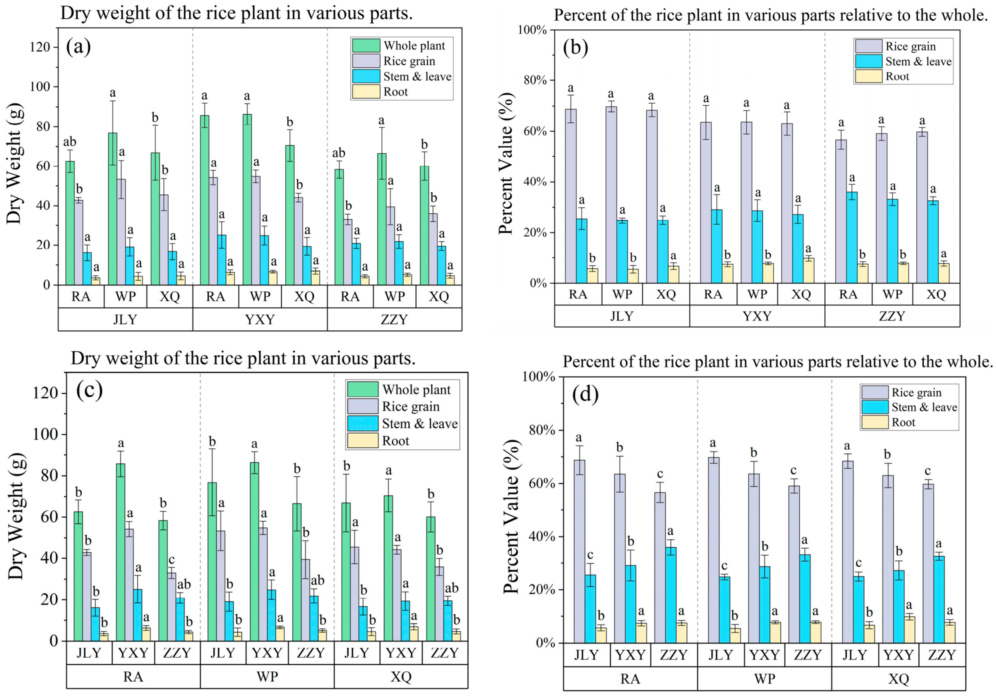

Figure 5. Analysis of rice biomass in the pot experiments. The abbreviations JLY, YXY, and ZZY refer to the names of the three rice varieties. The proportion of dry matter in different parts of the rice plant relative to the whole plant was calculated as follows: = (dry weight of various parts of the rice plant/dry weight of the whole rice plant) × 100%. The significance of differences between data was tested using Duncan's method ($p < 0.05$). The data were classified according to rice variety, and differences in data for the same rice plant parts across the three soil types were compared. The differences are indicated in the figures by lowercase letters. If the same letter does not appear in the markings between the comparison data, it signifies a significant difference.

3.3. Soil Physicochemical Properties during the Rice Growth Phase

In all soil types, the pH (Figure S1a) and SOM contents (Figure S1b) initially decreased and then increased during the rice growth process. The CEC (Figure S1c) exhibited no clear trend throughout the growth stages in any soil type, while the E_h (Figure S1d) declined before the heading stage, followed by divergent trends. These indicators displayed distinct levels of phase variation (Table S6). A multivariate ANOVA was performed on the data for these four indicators, using rice variety and growth stage as grouping variables. The results indicated that the growth stage of rice was the primary factor influencing changes in soil physicochemical properties across all soil types ($p < 0.05$). This variation is related to water management practices at different growth stages of rice, with soil physicochemical properties being crucial factors in determining the bioavailability of heavy metals.

3.4. Metabolic Characteristics of Heavy Metals and Nutrient Elements in Rice Plants across Different Growth Phases

To elucidate the metabolic characteristics of various heavy metals in rice plants during two critical growth phases (phase 4 and phase 5), differences in the bioconcentration factors (BCFs) (soil → root) and translocation factors (TFs) (root → grain) of different soil heavy metals were compared among three rice varieties under identical soil conditions (Figure 6).

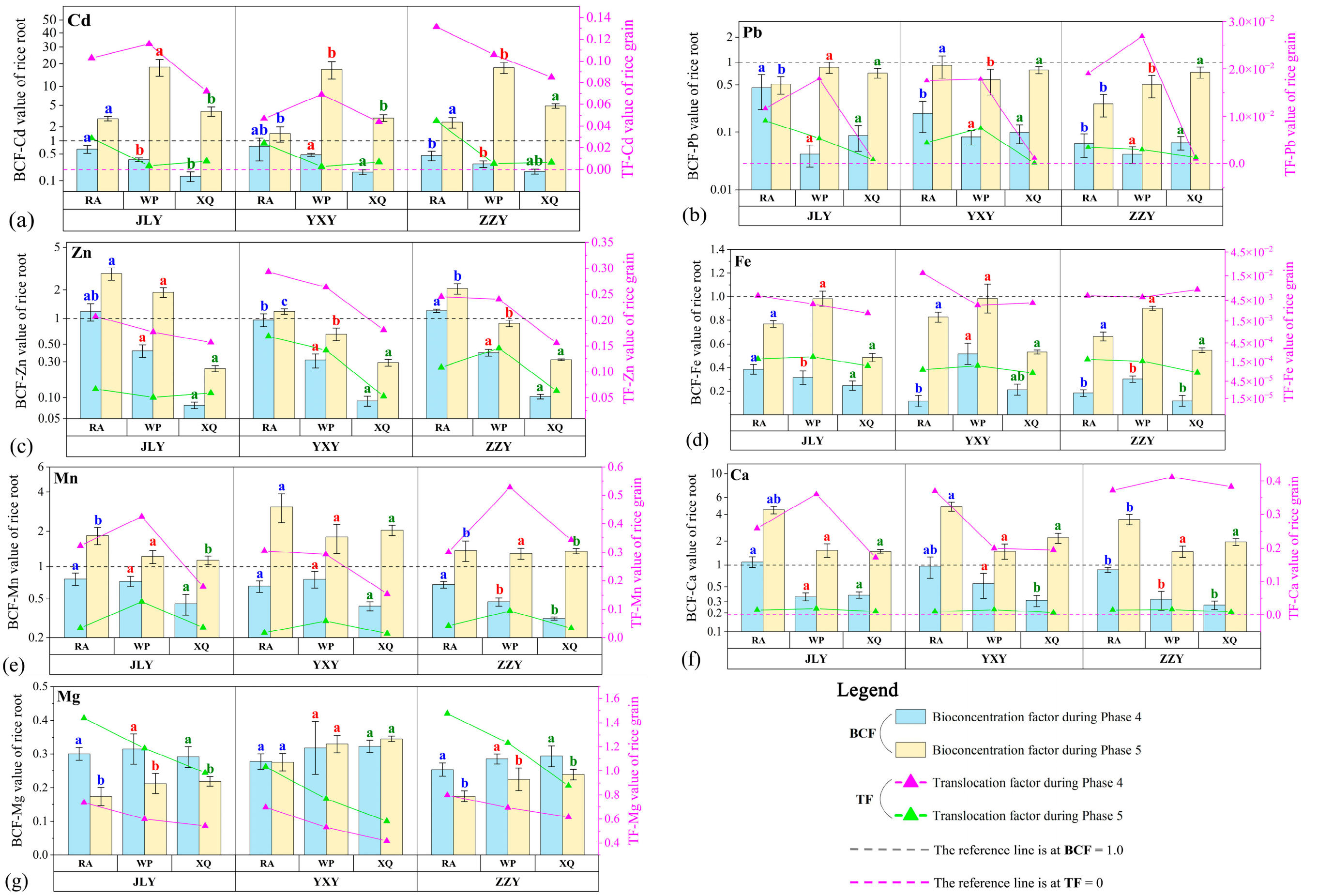

Figure 6. Data characterization of the BCF and TF at different growth phases. (**a**–**g**) illustrate the differences in data between the three rice varieties in terms of BCFs and TFs for Cd, Pb, Zn, Fe, Mn, Ca, and Mg across two growth stages (Phase 4 and Phase 5), all under the same soil conditions. Statistical significance of differences between data was assessed using Duncan's method ($p < 0.05$). Data were classified by soil collection location, and BCF and TF differences among the three rice varieties in the same soil were compared. Labels with the same color denote data from the same soil type. The differences are indicated in the figures by lowercase letters. If the same letter does not appear in the markings between the comparison data, it signifies a significant difference.

In rice plants, the translocation of Cd, Zn, Fe, Mn, and Ca exhibited higher levels during phase 4 compared to phase 5. In the RA and WP soils, the translocation of Pb was higher during phase 4 than in phase 5. However, in the XQ soil, there was no significant difference in the translocation of Pb among the three rice varieties, suggesting that the high Cd soil environment may have hindered the translocation of Pb. Additionally, the translocation of Mg showed higher ability during phase 5 than phase 4, and with the intensification of soil Cd stress on rice, the ability of rice to transport Mg was notably inhibited.

Analyses of root absorption of Cd, Pb, Zn, Fe, Mn, Ca, and Mg from the soil revealed that these elements began to accumulate as early as phase 4 and continued to accumulate until phase 5. However, Mg did not exhibit further accumulation, indicating that the growth and metabolic processes in rice had a stable demand for Mg.

The rankings of BCFs and TFs for various heavy metals in rice at phase 5 across different soil types are summarized in Table S7. Compared to the BCF rankings, the TF rankings for rice variety JLY may be influenced by the rice genotype. The BCFs of rice in WP soil were most affected, followed by RA soil, while XQ soil exhibited the least susceptibility, potentially due to the high Cd stress. A multivariate analysis of variance (ANOVA) conducted with rice variety and soil type as categorical variables revealed that soil type significantly affected the bioconcentration ability of different metals in rice roots ($p < 0.05$). In light of these findings, it can be inferred that the impact of varietal differences in rice on the mechanism of metal element metabolism is also constrained.

3.5. Interactions in Rice's Heavy Metal Metabolism

The processes involved in the accumulation of heavy metals from soil in rice and the interactive relationships during their internal transport are intricate. These elements may display antagonistic (opposing) or synergistic (cooperative) effects on each other. To investigate these dynamics, the metabolic indicators of rice plants were categorized by growth phase and variety. Subsequently, a correlation analysis was conducted between the TFs of Cd and Pb and the BCFs of Cd, Pb, Zn, Fe, Mn, Ca, and Mg (Figure 7). The results indicate that the physiological metabolism of Cd and Pb undergoes cyclic changes during rice growth, and significant interspecific differences exist.

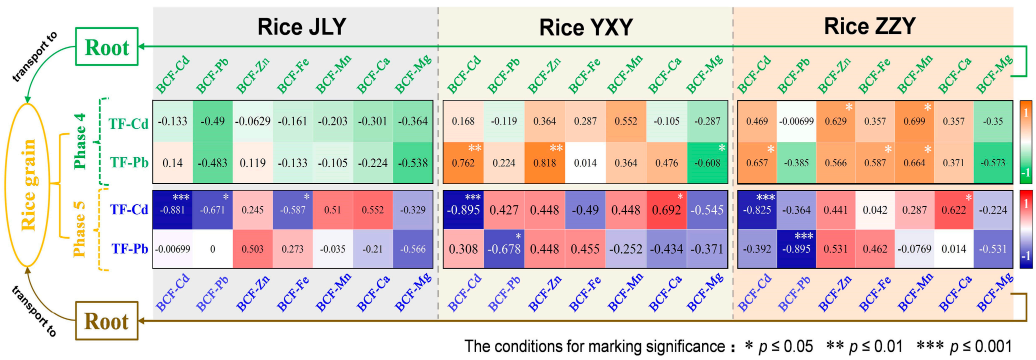

Figure 7. Interaction of heavy metal metabolic processes between grain and root in rice across various growth phases. Phase 4 corresponds to the full-heading stage, and phase 5 corresponds to the full-ripe stage. The abbreviations JLY, YXY, and ZZY represent the three rice varieties examined.

3.5.1. Interaction between Cd and Pb Metabolism in Rice Plants

From phase 4 to phase 5, there was a significant change in the correlation between TF-Cd and BCF-Cd in all rice varieties, shifting from non-significant to a significant negative correlation. Similarly, the association between TF-Pb and BCF-Pb in rice YXY and ZZY changed from a non-significant to a significant negative correlation. These findings indicate that as soil Cd and Pb accumulate in rice during growth, the degree of stress imposed by these elements intensifies. Rice reduces the transport of hazardous elements from roots to aboveground parts through regulatory metabolism, thereby reducing organ damage. Additionally, the Pb tolerance of rice JLY ($r = 0$) was found to be greater than that of rice YXY ($p \leq 0.05$) and rice ZZY ($p \leq 0.001$).

The correlation between TF-Cd and BCF-Pb was compared in all rice varieties, with only rice JLY demonstrating a significant negative correlation ($p \leq 0.05$) in phase 5. This suggests that continuous accumulation of Pb in the roots of rice JLY from the soil may inhibit the transport of Cd from roots to grains, resulting in antagonistic effects. Previous studies have indicated that Pb prevented Cd from moving from rice roots to aboveground parts of the plant, thereby reducing Cd levels in the grains [68].

Furthermore, in rice YXY and ZZY, the correlation between TF-Pb and BCF-Cd shifted from significantly positive to non-significant. However, in rice JLY, this correlation was never significant. This indicates that the interaction of Cd and Pb in the roots of rice YXY and ZZY may enhance the translocation of Pb from roots to grains until phase 4. Therefore, it can be concluded that the metabolic pathways of Cd and Pb in rice plants are similar, and the result observed in rice JLY can be attributed to rice genotypes.

3.5.2. Interaction of Cd and Pb Metabolism with Nutritional Elements in Rice Plants

During the growth of rice JLY, the relationship between root-to-grain translocation of heavy metals (Cd and Pb) and root absorption of soil nutrients (Zn, Fe, Mn, Ca, and Mg) differed from other varieties. In phase 5, TF-Cd was the only one showing a significant negative correlation ($p < 0.05$) with BCF-Fe. This indicates that Fe enrichment in rice JLY roots significantly inhibited Cd transfer from roots to grains, resulting in an antagonistic effect. Since Fe is primarily transported in plant tissues via chelates, the antagonistic relationship between Fe and Cd suggests competition for binding sites. Additionally, rice plants oxidize reducible chemicals in waterlogged soils through radial oxygen loss (ROL) from leaves to roots, producing a gel-like membrane on the root's surface primarily composed of Fe and Mn oxides. This barrier limits the uptake of hazardous ions by roots through adsorption and co-precipitation [69,70].

This study revealed a shift in correlation from significant to non-significant between TF-Pb of rice YXY and BCF-Zn and BCF-Mg at both growth phases. Some scholars have suggested a lack of direct correlation between soil nutrient uptake by roots during the grain-filling stage in rice [41], negating a substantial link with phase 5. This suggests that, before phase 4, the enrichment of Zn and Mg in rice roots had a significant impact on Pb transfer from roots to ears. Soil Zn enrichment improves Pb transport from roots to ears in rice ($p < 0.01$), indicating a synergistic effect of BCF-Zn on TF-Pb. Enriching soil Mg in rice roots limits Pb transport from roots to ears ($p < 0.05$), indicating an antagonistic effect of BCF-Mg on TF-Pb.

During phase 4, rice ZZY roots accumulated Fe, and Mn was significantly positively correlated ($p \leq 0.05$) with TF-Pb transport. Meanwhile, its roots accumulated Zn, and Mn showed the same correlation ($p \leq 0.05$) with TF-Cd transport. However, both were not significantly correlated when entering phase 5. These results suggest that Cd and Pb may share the same metabolic pathway in rice ZZY. Previous research has shown that the Os*Nramp*5 transport protein on the outer plasma membrane of rice root cortical cells may absorb Mn and Zn while delivering Cd^{2+} into the root cells [71,72].

As rice YXY and ZZY reached phase 5, there was a substantial positive correlation ($p < 0.05$) between TF-Cd and BCF-Ca. This indicates that Ca absorbed in rice roots facilitates Cd translocation into rice grains. Previous research has demonstrated a strong connection between Ca levels in rice grains and protein content during the filling stage [73]. These findings suggest a potential homology between Ca in rice roots and Cd in rice grains.

3.6. Key Factors Controlling Heavy Metal Content in Rice Grain

By substituting the primary control factors identified in the screening into Equation (4), theoretical models were developed to explain soil-induced enrichment of Cd and Pb in rice roots and their subsequent transport to rice grains, as illustrated in Figure 8.

Figure 8a,c illustrates theoretical models of Cd content in rice grains constructed with the following key control factors:

- In the WP soil, soil Cd bioactivity was enhanced and accumulated in rice roots due to pH regulation and chelation with SOM. In contrast, the interaction between soil Cd and soil Mg reduced Cd accumulation in rice roots. In the XQ soil, soil Ca enhanced Cd accumulation in rice roots by chelating with SOM, but the interaction between soil Zn and soil Mg inhibited Cd accumulation in rice roots. The influence of SOM [6] and soil Mg on Cd accumulation in rice roots was similar in both soil types;
- In the WP soil, Cd transport from rice roots to aboveground organs causes continuous Cd accumulation in plants, resulting in a certain level of Cd stress. During this stage, the rice plant employs metabolic regulation to minimize the transfer of Cd to the grains. The physiological stress in the plant escalates with the accumulation of Cd. Furthermore, the interaction between Ca and Fe in rice roots inhibits Cd translocation from the roots to the grains. In the XQ soil, Fe in rice roots inhibits the transfer of Cd from roots to grains through metabolic pathways. The findings showed that the mechanism of Fe in rice roots for Cd translocation from roots to grains was similar in both soil types [6,74].

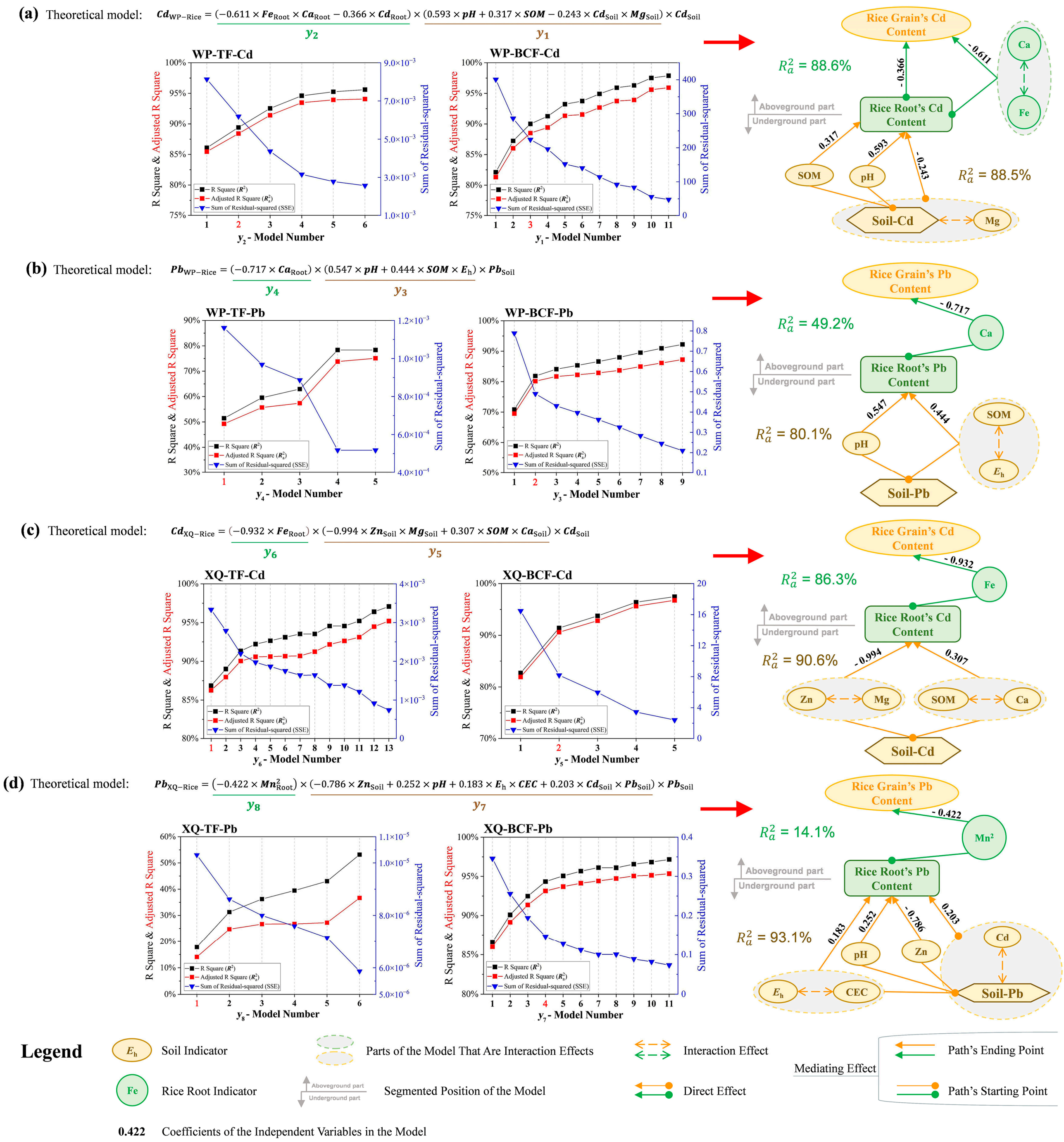

Figure 8. Screening results for the main controlling factors of heavy metal contents in rice grain. The stepwise regression conditions were set as follows: $\alpha_{entry} = 0.35$; $\alpha_{removal} = 0.65$. The screening outcomes align with the least squares method: $n > p + 1$ [64], where n represents the number of samples, and p indicates the number of independent variables. The R squared represents the multiple coefficients of determination (R^2), while the adjusted R squared represents the adjusted multiple coefficients of determination (R_a^2). The optimal subset of the models, which has been filtered, is indicated by the red numbers marked on the x-axis. The model results for each soil type are based on standardized coefficients.

Figure 8b,d illustrates theoretical models of Pb content in rice grains constructed with the following key control factors:

- In the WP soil, soil Pb bioactivity is enhanced and accumulated in rice roots due to pH regulation and chelation with SOM in the soil E_h. In the XQ soil, soil Pb bioactivity and accumulation in rice roots were enhanced due to pH regulation, the E_h influence on CEC, and interaction with soil Cd. In contrast, soil Zn inhibited the accumulation of soil Pb in rice roots. The findings show that the mechanisms of E_h and pH control effects on soil Pb accumulation in rice roots were similar in both soil types. Furthermore, the interaction effect of soil E_h on SOM (or CEC) suggests the proliferation and increase in soluble organic matter in soils under reducibility conditions, indirectly enhancing the biological activity of soil Pb;
- In the WP soil, Ca in rice roots was found to inhibit Pb translocation from roots to grains through metabolic pathways. In the XQ soil, Mn in rice roots may inhibit Pb translocation from roots to grains through metabolic pathways, with a nonlinear relationship observed. The findings show that the indicators filtered from rice roots had a considerably limited ability (R_a^2, TF < BCF) to explain the Pb translocation from roots to grains in both soil types. This suggests that the indicators linked with rice roots can only partially explain the transfer of Pb from roots to grains. As a result, it is necessary to expand the scope of relevant indicators.

3.7. Chemical Speciation Analysis (BCR) of Soil Heavy Metals

The chemical speciation of soil heavy metals is a critical factor in determining their bioavailability to rice roots. Additionally, the fundamental physicochemical properties of the soil can directly affect the transformation of soil heavy metal chemical forms. Soil environmental conditions in paddy fields are significantly influenced by water management practices (moisture changes) during rice cultivation. Therefore, studying the speciation (BCR) of Cd and Pb in soil, as well as their bioavailability to rice roots, is essential for understanding their impact on rice cultivation (Figure 9).

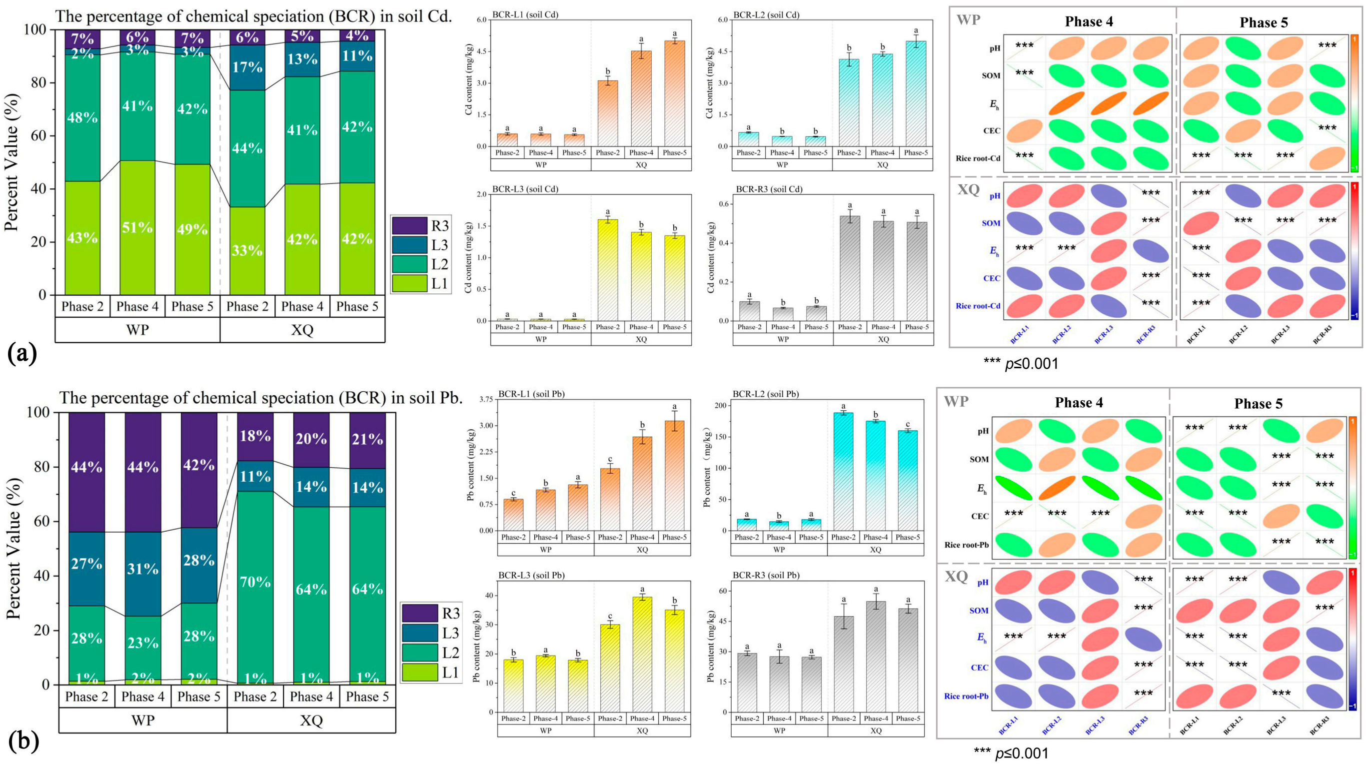

Figure 9. Chemical speciation analysis (BCR) of Cd and Pb in soil during rice cultivation. (**a**) presents data on the different chemical speciation of soil Cd during the three critical growth phases of rice, the differential changes in each speciation, and between the growth phases and the correlation between

the basic physical and chemical properties of the soil, the Cd content in rice roots, and the chemical speciation of soil Cd. (**b**) presents data on the different chemical speciation of soil Pb during the three critical growth phases of rice, the differential changes in each speciation, and between the growth phases and the correlation between the basic physical and chemical properties of the soil, the Pb content in rice roots, and the chemical speciation of soil Pb. Phase 2 corresponds to the rice transplanting stage, phase 4 to the full-heading stage, and phase 5 to the full-ripe stage. The abbreviations JLY, YXY, and ZZY refer to the names of the three rice varieties. The significance of differences between data was tested using Duncan's method ($p < 0.05$). The data were classified according to rice growth phase and compared within each soil type. The differences are indicated in the figures by lowercase letters. If the same letter does not appear in the markings between the comparison data, it signifies a significant difference.

3.7.1. Chemical Speciation Analysis of Soil Cd

Figure 9a illustrates the chemical speciation of soil Cd during key phases of rice growth, highlighting the variability between different phases and their effects on the bioavailability of Cd in rice roots. The experimental results showed that the L1 and L2 contents of Cd in both soil types accounted for a larger proportion, increasing the risk of exceeding the Cd content in rice.

Significant changes were observed in the L2 and R3 contents of Cd in WP soil across different rice growth phases. The L2 content decreased significantly in phase 4 and then remained stable in phase 5. The R3 content exhibited a similar pattern of change, likely related to the prolonged inundation of the soil. The decrease in R3 content was likely due to soil saturation with water. In XQ soil, significant differences were noted in the L1, L2, and L3 contents of Cd. The L1 content in phase 4 increased significantly and then sta-bilized in phase 5, while the L3 content in phase 4 decreased significantly. The opposite patterns of change between L1 and L3 suggest potential among both speciation interconversions. In soils with high SOM concentration, Pb's competitive impact increases the amount of Cd in the acid-soluble fraction [33]. The L2 content did not change significantly in the first two phases but increased significantly in phase 5, possibly related to the cessation of flooding at the maturity stage. The R3 content did not differ significantly across the three phases [75].

Correlation analysis indicated that the Cd content of rice roots in WP soil was significantly and negatively correlated with the L1 content of soil Cd in phase 4. Additionally, soil pH significantly increased the L1 content, while SOM significantly inhibited L1 formation. In phase 5, the Cd content of rice roots showed highly significant correlations with the L1, L2, and L3 contents of soil Cd. L1 and L3 demonstrated positive correlations, while L2 exhibited negative correlations. These correlations may be associated with metabolic alterations and the accumulation characteristics of heavy metals in rice plants during rice growth. In XQ soil, the Cd content of rice roots exhibited a positive correlation with the L1 content of soil Cd in phase 5. Additionally, soil pH, soil E_h, and CEC showed strong correlations with the L1 content of soil Cd. However, due to changes in water management practices during this phase, accurately determining the influence of soil physicochemical properties on L1 was not feasible.

3.7.2. Chemical Speciation Analysis of Soil Pb

Figure 9b illustrates the chemical speciation of soil Pb during key phases of rice growth, highlighting the variability between different phases and their effects on the bioavailability of Pb in rice roots. The experimental results showed that the L1 content of Pb in both soil types did not exceed 2%. Furthermore, WP soil exhibited the highest percentage of R3 content of Pb, while the percentages of L2 and L3 content were comparable. In contrast, XQ soil demonstrated the highest percentage of L2 content of Pb, reaching over 60%.

Changes in the L1 content of Pb in WP and XQ soils were consistent across different rice growth phases, with a significant increase observed from phase 2 to phase 5. The trends of changes in the L2 and L3 contents of Pb in both soil types were similar, with a significant decrease in L2 content in phase 4 and a significant increase in L3 content in phase 4. This suggests potential interconversion between various speciation forms. In phase 5, however, differences in the trends of changes in L2 and L3 contents of Pb in the two soil types may be related to water management practices during that phase. The R3 content of Pb in both soil types exhibited no significant differences across the three phases.

Correlation analysis indicated that the Pb content of rice roots in WP and XQ soils was significantly and positively correlated with the L3 content of soil Pb, with this correlation being exclusive to phase 5. This result may be associated with metabolic changes occurring during the rice plant's growth process.

4. Discussion

4.1. Rice Yield Reduction under High-Cd Soil Stress

As described in Section 3.2 on Rice Growth Status, rice yields significantly decreased under high Cd stress (XQ), while no notable differences were observed in other plant organs. This indicates that Cd accumulation continued in various rice organs before reaching the full-heading stage without exceeding tolerance limits. During the reproductive growth stage, the physiological metabolism responsible for heavy metal transport from roots to grains slowed. However, Cd has accumulated in various organs of rice over time, and at this point, it may have reached the tolerance limit of rice physiology and caused some physiological damage to rice. Cd and Pb accumulation in early growth stages leads to increased production of free radicals (O^{2-}, H_2O_2), causing oxidative damage, inhibiting chlorophyll synthesis, and disrupting key enzyme activities involved in light energy conversion [76]. At this stage, stress severity directly affected rice grain yield. Rice grain formation involves complex interactions among carbon, nitrogen, and lipid metabolism, closely linked to photosynthetic product synthesis, grain filling, and key enzyme activities [77].

The rice variety YXY demonstrated remarkable productivity in the high geologic background of Cd levels, as observed in the WP and XQ soil types. Additionally, results from the pot experiment (Section 3.1) showed that in WP soil, the Cd content of rice YXY was significantly lower than that of the other two varieties. Furthermore, in the presence of combined Cd and Pb pollution in the XQ soil, the Cd and Pb contents of rice YXY were significantly lower than those of the other two varieties. This implies that rice YXY may have the potential for low Cd accumulation.

4.2. SOM Increases the Risk of Exceeding Pb Levels in Rice Grain

Previous research has found that Pb has a greater affinity for SOM surfaces than Cd. This could be due to Pb's larger ionic radius, lower hydration, and greater likelihood of forming inner-sphere complexes with oxygen-containing functional groups in the soil, leading to more stable bindings [78,79]. This gives Pb a competitive advantage in coexisting systems. Since SOM is the principal component responsible for Cd and Pb adsorption, the competitive impact of Pb on Cd is stronger in soils with high SOM concentration and less so in soils with low SOM concentration [33]. As shown in Table S1, the SOM content was similar between WP and XQ soils, approximately double that of the RA soil (control). The exceedance rate of Pb levels in rice grain was 83.3% for WP soil and 50% for XQ soil, indicating high Pb bioavailability in these soils. Additionally, a highly significant positive correlation was observed between the oxidizable fraction (L3) of Pb in WP soils and the Pb content in rice roots. This suggests that Pb in the soil may bind with organic reactive groups, enhancing its bioavailability. Previous studies have indicated that SOM can increase the solubility and bioavailability of soil Pb [80–85]. The theoretical modeling of Pb content in WP soil also suggested that SOM enhanced Pb uptake by rice roots when influenced by soil E_h.

4.3. Water Management Measures Directly Affect the Bioavailability of Soil Heavy Metals

Starting from the full-heading stage of rice plants, the soil was no longer submerged in water in the pot experiment. The research findings suggest that alterations in water management practices resulted in modifications in the fundamental physical and chemical properties of the soil during the rice growth period. In paddy soil, soil-E_h related to water management essentially controls metal solubility [80]. Yang et al. [86] investigated the effects of different irrigation methods on the uptake of Cd and arsenic by two rice varieties under diverse soil moisture conditions, demonstrating that prolonged flooding effectively reduced the Cd contents in rice plants. Therefore, the variation in soil moisture content led to changes in the physicochemical characteristics of the soil, thereby influencing the bioavailability of soil heavy metals [87,88]. For Pb, alternate wetting and drying have been demonstrated to effectively reduce Pb accumulation in rice compared to continuous ponding [89]. In a previous study, flooding reduced the Cd in rice grain by 92% in acidic soil, while minor changes were found in slightly alkaline soil [90].

4.4. The Limited Efficacy of Reducing Heavy Metal Contents in Rice Grain through Rice Variety

Under no or low Cd stress, the metabolic mechanism of different heavy metals within rice is primarily self-regulated, with interspecific (genotypic) differences observed. The difference in transport ability for various heavy metals was greatest between rice JLY and the others, although only for a few elements. Meanwhile, the accumulation ability for different heavy metals in rice roots differed significantly among the three varieties, particularly in the WP soil. However, under high-Cd stress (XQ soil), the metabolic mechanism of heavy metals within rice plants of the three varieties is primarily influenced by soil physicochemical properties, with no discernible differences. The overall effect of pot experiments indicates that inhibiting Cd and Pb bioconcentrations in rice grains through rice varieties was limited.

4.5. The Interactive Effects of Metabolism between Different Heavy Metals in Rice Plants

At maturity, the three rice varieties showed significant negative correlations between TF-Cd and BCF-Cd, as well as between TF-Pb and BCF-Pb. This finding contrasts with previous studies that reported a positive linear relationship between Cd and Pb concentrations in rice grains and roots [91]. Those earlier studies mainly described the correlation between Cd and Pb accumulation across different rice organs. However, our study focuses on analyzing the uptake of Cd and Pb from the soil by rice roots at various growth stages and their subsequent transport to the rice grain.

Nutrient elements absorbed by the roots significantly influence the metabolism of Cd and Pb within rice plants. Under heavy metal stress, plants employ avoidance mechanisms to minimize uptake and metabolic mechanisms to reduce accumulation and mitigate toxicity. Our findings also revealed significant periodic differences in the metabolic processes involved in the translocation of Cd and Pb to rice grains, influenced by interactions with other heavy metals. These insights can inform agricultural measures aimed at regulating Cd and Pb content in rice grains.

4.6. Theoretical Models of Cd and Pb Contents in Rice Grains Based on Metabolic Mechanisms

Previous research has shown that the accumulation of Cd and Pb in rice grains is not directly correlated with their total concentrations in soil. Instead, this process involves a complex interplay of components and interactions with other elements [92,93]. The theoretical models developed for Cd and Pb content in rice reflect these complexities. These models suggest that the biological activities of soil Cd and Pb are influenced by the soil environment, which, in turn, regulates their absorption by rice roots. Additionally, these models demonstrate the presence of mediating and interaction effects between independent and dependent variables.

Except for the XQ soil model of Cd content in rice grains, the coefficient of determination for the bioenrichment model (root uptake) was significantly higher than that for the translocation model (aboveground parts) in all other models. This indicates that heavy metal uptake by rice roots is largely influenced by the physicochemical properties of the soil. In contrast, the translocation of heavy metals in the aboveground parts, which is crucial for controlling heavy metal movement, is likely influenced by other variables related to metabolic regulation. Previous studies have indicated that differences in the ability of plants to regulate Cd transfer from the xylem to the phloem and, subsequently, to the grains may contribute to variations in Cd concentrations in rice [94]. To improve the explanatory power of the theoretical model for the aboveground transport of heavy metals, it is necessary to incorporate additional factors that regulate heavy metal metabolism. However, the specific factors to be included in the model remain unclear and require further investigation.

These theoretical models provide a detailed framework for understanding the primary variables and their relative importance in influencing Cd and Pb accumulation in rice grains, both aboveground and underground. They also offer a practical foundation for strategies aimed at regulating heavy metal accumulation in rice grains.

4.7. Theoretical Modeling of Rice Cd and Pb Content Corresponding to Their Soil Chemical Specification

Theoretical models have identified various mediating and interacting effects influencing rice roots' performance in the uptake of soil heavy metals. These mechanisms are closely related to the chemical speciation of soil heavy metals, which predominantly dictates their bioavailability [95].

During rice root flooding cultivation, the prolonged reducing environment (E_h) before the tasseling stage promotes the proliferation of organic components in the soil. Decaying biological residues contribute to the increase in SOM. The activation of soil metals by dissolved organic matter (DOM) is a crucial mechanism leading to the uptake of heavy metals by rice roots [96], as reflected in all theoretical models.

In this study, we validated and analyzed the theoretical model of rice Cd content in WP soil in conjunction with experimental results from two stages, phase 4 and phase 5, as illustrative examples. Analyzing the chemical speciation of soil Cd and Pb revealed a highly significant positive correlation ($p \leq 0.001$) between the Cd and Pb content in roots and BCR-L3 in rice grown in WP soil, supporting the theoretical model. However, while the Pb content in XQ rice roots also showed a highly significant correlation with BCR-L3, the effect was opposite. It is inferred that a high Cd environment led to competition for organically bound Pb sites by Cd^{2+}, promoting the uptake of soil Cd^{2+} by rice roots.

In WP soil, Cd uptake by rice roots was regulated by soil pH. A highly significant negative correlation between soil Cd BCR-L1 (phase 4) and rice root Cd content confirmed this. Soil pH before the flush stage showed a significant decreasing trend from transplanting, and rice root Cd content gradually accumulated during this period. As soil pH decreased, soil Cd BCR-L1 became an important Cd source for rice root uptake, consistent with the physiological performance of rice roots before the flush stage. BCR-L1 (phase 5) showed a highly significant positive correlation with rice root Cd content, aligning with the characteristics of heavy metal accumulation in rice roots.

The theoretical model suggests an antagonistic relationship between soil Cd and Mg, inhibiting soil Cd accumulation in rice roots. BCR-L2 (phase 5) showed a highly significant negative correlation with rice root Cd content at maturity, consistent with soil Mg's antagonistic performance. Under reducing conditions of soil flooding, Cd (BCR-L2) adsorbed in the Fe-Mn oxide-bound state is dissolved and released due to the reduction in Fe^{3+} to Fe^{2+}. Soil Mg^{2+} competes with Cd^{2+} for uptake by rice roots, inhibiting Cd^{2+} uptake and resulting in an antagonistic effect.

4.8. Focus on the Metabolism between Mg and Cd (or Pb) in Rice Plants

Mg plays a crucial role in numerous physiological and metabolic processes essential for plant development. It is a vital component of chloroplast molecular structures, with approximately 75% of Mg^{2+} in leaves involved in protein synthesis. Additionally, Mg acts as a catalyst for various enzymes in plant cells.

In this study, the translocation factor for Mg (TF-Mg) was higher at the full-ripe stage compared to the full-heading stage, indicating a significant demand for Mg during ripening.

Some studies suggest that plant Mg absorption occurs in two phases: an initial phase characterized by a high absorption rate within the root zone, followed by a subsequent phase of slower, stabilized absorption [97]. During the grain-filling period, Mg^{2+}-containing Rubisco (ribulose-1,5-bisphosphate carboxylase oxygenase) regulates the Calvin cycle of the dark reaction in photosynthesis, facilitating the synthesis of sugars and starch [98]. Notably, Mg did not accumulate in rice roots from the full-heading stage to the mature stage, a pattern similar to that observed for copper in a soil–Brassica *rapus* system [99].

The transport of Cd and Pb from roots to ears in rice plants is negatively correlated with Mg enrichment in roots at different stages. However, only in the YXY rice variety is the TF-Pb significant at the full-heading stage ($p \leq 0.05$). Moreover, in the theoretical model, Mg appears in the BCF component for both the WP and XQ soils, indicating that Mg primarily influences Cd accumulation in rice grains through root absorption.

Although limited research has examined Mg metabolism in growing rice plants under soil Cd (or Pb) stress, this area warrants further investigation. Such research could provide valuable insights into reducing heavy metal content in rice grains.

5. Conclusions

In this study, we developed a segmented regression model to elucidate the levels of Cd and Pb in rice grains. This model was based on the metabolic pathways of rice plants in response to soil heavy metals and the structural characteristics of polynomial regression equations. Using stepwise regression, we identified key factors influencing both the bioconcentration factor (BCF) and the translocation factor (TF) in rice plants. Subsequently, we constructed a theoretical model that incorporated these principal-regulating variables. This model provides a comprehensive framework for predicting heavy metal levels in rice grains, elucidating the partial metabolic process of soil heavy metals from rice roots to grains. The ultimate goal is to provide a theoretical basis for implementing safe and reliable interventions in local rice cultivation to reduce Cd content in rice grains, thereby informing soil testing and formula fertilization.

Our findings reveal that the key factors controlling Cd and Pb levels in rice grains can be categorized into two main components: (i) factors affecting accumulation in rice roots and (ii) factors governing transport from rice roots to grains. We observed that using soil physicochemical indicators to explain Pb accumulation in rice roots was more effective than models focusing on Pb translocation from roots to grains. Pot experiments demonstrated that differences among rice varieties did not significantly affect the transport of Cd, Pb, Zn, Fe, Mn, Ca, and Mg in the aboveground parts of the plants under identical soil conditions. Thus, the potential for controlling Cd bioconcentration through rice varieties appears limited.

This research underscores the critical need to monitor Pb levels in rice, especially in karst geologic areas with high soil Cd concentrations. Elevated levels of SOM may contribute to Pb accumulation in rice grains that exceeds regulatory limits. Additionally, our study highlights significant differences in the metabolic patterns of Mg compared to other elements during rice growth. We found that soil Mg antagonized Cd enrichment in rice roots. Furthermore, this study suggests that the rice variety YXY may have the potential for low Cd accumulation.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14092076/s1, Table S1: Physics and Chemistry Characteristics of Test Soil; Table S2: Pot Experiment Grouping; Table S3: Basic Form of the Metabolic Mechanism Model (second-order polynomial); Table S4: Results of Correlation and Fitting in the Test Indicator; Table S5: Screening Results of Stepwise Regression; Table S6: Soil Physicochemical Index During the Critical Phase of Rice Growth; Table S7: Metabolic Properties of Various Metals in Rice Plants at the Maturation Stage; Figure S1: Basic Physicochemical Index of Test Soil During the Rice Growth Process.

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