5-day flooding followed by 3-day drainage wet-dry alternation cycle is highly effective in reducing Cd and As content in rice

Qiongli Bao (✉ qionglibao1982@163.com)

Agro-Environmental Protection Institute, Ministry of Agriculture and Rural Affairs

Wankui Bao

Institute of Agricultural Resource and Regional Planning, China Academy of Agricultural Sciences

Yan Li

Agro-Environmental Protection Institute, Ministry of Agriculture and Rural Affairs

Shengnan Zhang

Agro-Environmental Protection Institute, Ministry of Agriculture and Rural Affairs

Yizong Huang

Agro-Environmental Protection Institute, Ministry of Agriculture and Rural Affairs

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Abstract

To comparatively and simultaneously investigate the reducing efficiency of the different wet-dry alternation cycles on the Cd and As absorption and transportation in rice organs, and synthesis of amino acids (AAs) in rice in two soils with different levels of Cd and As contamination, controlled pot experiments were conducted in this study. Results showed that wet-dry alternation treatments reduced Cd and As concentrations in grains by 18.8%-80% and by 77.4%-86.7% in W soil, respectively; and 76.1%-90.8% and 73.1%-80.6% in H soil, respectively. Cd and As concentrations in the soil solution were negatively and positively correlated with soil pH, respectively; but positively and negatively correlated with soil redox potential (Eh), respectively. The minimum “trade-off” values were observed in the 5 d Flooded followed by 3 d Drained treatment in both soils. The Drained treatment greatly improved AAs contents in rice organs in both soils. The changes of AAs were negatively explained more than 70% by the rachises As. Totally, the F5D3 treatment was identified as the optimal measure for simultaneously minimizing Cd and As accumulations in rice in studied soils, and water management regimes regulated the synthesis of AAs in rice organs by affecting the accumulation of As in rachises.

Introduction

Cadmium (Cd) is a highly toxic heavy metal. Because its effects on animals are cumulative, it is toxic to livestock and humans at lower concentrations than to plants. Compare with other heavy metals, Cd was found to be the main components contributing to the potential health and ecological risks in China by the latest studies^{1,2}. The Joint Expert Committee on Food Additives (JECFA) has established a health-based guidance value for Cd ($25 \mu\text{g kg}^{-1}$ bodyweight per month)³, and the Codex Alimentarius Commission has adopted a maximum concentration of 0.4 mg kg^{-1} for Cd in white rice grain⁴. As is a carcinogen harmful to the human body. As exists in soil in many chemical forms, including inorganic As and methylated As species like monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). As mainly exists in the form of inorganic As in soil and is taken up by plants easily⁵. It is noteworthy that rice provides the greatest source of dietary intake of inorganic As in China⁶. A provisional tolerable weekly intake of $15 \mu\text{g kg}^{-1}$ body weight has been set by the JECFA. The national food standard limits of Cd and As are 0.2 mg kg^{-1} for rice in China⁷.

Numerous studies have found that water management influences the bioavailability of Cd^{8,9} and As¹⁰ in soils. When the soil is flooded (the soil has a low redox potential), any Cd and sulfur (S) in soil combine to form CdS. The solubility of CdS in water is low⁸, which reduces the absorption of Cd by plants¹¹. When the soil is drained (oxidative condition), CdS is converted into CdSO₄ (through oxidation of CdS to Cd²⁺ and SO₄²⁺), which enhances the availability of Cd for plant absorption⁸ because CdSO₄ has much higher solubility than CdS. On the contrary, under drainage conditions, As(V) is the predominant inorganic As species and is easily combined with Fe and Al (hydr)oxides¹², thereby decreasing its mobility and is not easily absorbed by plants. When soil is flooded, As is released from the solid phase into the aqueous

solution phase by dissolution of Fe and Al(hydr)oxides and reduction of As from As (V) to As (III), which has enhanced solubility compared with As (V)⁵, and is easily taken up by plants.

Cd and As bioavailability and solubility are regulated by the alternation of Eh and pH in soil, and primarily determines the content of Cd and As in rice grains. Accordingly, the content of Cd and As in grains can be controlled through water management during the growing stage. Several studies confirmed that under wet-dry alternation conditions, a trade-off relationship exists between Cd and As bioavailability that regulates the accumulation of Cd and As in grains^{13,14,15}. Honma et al. (2016)¹⁵ found that the intermittent wet-dry alternation of 3 d Flooded followed by 5 d Drained treatment was effective for simultaneously reducing the content of Cd and As in grains.

Until now, a comparative and simultaneous study on the reducing efficiency of the different wet-dry alternation cycles of the Cd and As absorption and transportation in rice organs, and on the synthesis of AAs in rice (AAs are the building blocks of protein, and rice protein is a rich source of lysine, leucine, isoleucine, and glycine. In humans, when AAs are consumed less than the recommended daily intake, deficiency symptoms can be observed) in both high and low compound polluted soils is still scarce. In the present study, a pot experiment was conducted to investigate the simultaneous influences on Cd and As content and the synthesis of AAs in rice organs induced by different wet-dry alternation cycles. The treatments were performed from the elongation stage to the grain filling stage of rice in two soils.

Materials And Methods

Description of soil properties

Two paddy soils (0–20 cm) were collected from the rice field in the Wangcheng (abbreviated as W soil) district, in Changsha city, and in Helonghu town (abbreviated as H soil), Xiangyin county in Hunan province, China, in February in 2018. Two soils were typical heavy metal polluted paddy soils with different degrees of pollution from China. Air-dried and sieved to < 2 mm soils were prepared for the pot experiments. The soil characteristics are shown in Table S1 (Supporting information).

Plant cultivation and treatments

Seeds of conventional indica two-line hybrid rice (Tanliangyou 83) were chosen for the experiments. The cultivation process of rice seedlings was conducted following Feng et al. (2013)¹⁶. The sieved soils were transferred into 6 L PVC pots (5 kg of soil per pot). Tap water was added to pots on a daily basis to maintain the flooding condition for one month before transplanting rice seedlings, in which six plants were transferred into each pot. The pot cultivation was conducted in a greenhouse, where five different water measures were practiced in the pots from the elongation stage to the grain filling stage of rice: (1) Flooded, in which soil always remains flooded; (2) F5D3, in which wet-dry alternation cycle is repeated every 8 d, with 5 d Flooded followed by 3 d Drained treatments; (3) F5D5; in which wet-dry alternation cycle is repeated every 10 d, with 5 d Flooded followed by 5 d Drained treatments; (4) F5D7, in which wet-dry alternation cycle is repeated every 12 d, with 5 d Flooded followed by 7 d Drained treatments; (5)

Drained, in which soil always remains aerobic. Each treatment was performed with 4 triplicates. The basic P and K chemical fertilizers ($P_2O_5/K_2O = 1:1.5$) were applied at a rate of 0.06 g kg^{-1} of dry weight of soil, and urea application rate was 0.5 g kg^{-1} of dry weight of soil. The rice seedlings were transplanted on May 10, 2018. All of the pots were rearranged randomly each week until half a month before harvest. The rice was harvested on September 5 in 2019.

Sample collection and plant and soil analysis

The harvested rice plants were rinsed carefully and separated into roots, stems, leaves, rachises, and grains. Dried tissue samples were crushed to powder. Samples were digested according to Zhao et al. (2020)¹⁷. The As and Cd concentrations in the digested solution were determined using inductively coupled plasma mass spectrometry (Agilent 7500a, USA). The accuracy of the Cd and As analysis was confirmed using standard reference material [Cd standard solution (GSB04-1721-2004) and As standard solution (GSB04-1721-2004), respectively] from the Centre for Standard Reference of China.

Soil redox potential (Eh) was measured using an Eh meter (Mettler-Toledo, LED501 ORP, USA). Soil solution sampling was carried out on the last day of flooding or drainage of each cycle of wet-dry alternation by soil solution samplers (MOM Rhizon samplers, Netherlands). The total dissolved Cd and As concentrations in the soil solution was determined as described for the digested solution. The pH in the soil solution was determined immediately by a pH meter (Sartorius PB-10, Germany) after the soil solution was taken back to the laboratory.

We analyzed the amino acids (AAs) concentrations in the husks, stems, and roots of rice, as the rice grains were not sufficient. We have chosen samples for testing from the Flooded, D5F3, and Drained treatments. The quantification of AAs was performed as previously described by Zhao et al. (2020)¹⁷.

Statistical Analysis

All data were reported as mean $\pm$ SD. Multiple comparisons were conducted among different treatments ($\alpha = 0.05$). The relationships between soil indexes and plant variables were investigated by Pearson correlation analysis. SPSS version 21.0 (SPSS, USA) and SAS (SAS Institute, Cary, IN) software were used.

Results

Cd and As concentrations in rice organs

The highest and the lowest Cd concentrations in rice organs in both soils were observed in the Drained and Flooded treatments, respectively. In wet-dry alternation cycle treatments (F5D3, F5D5, F5D7), more drainage days resulted higher Cd concentration in organs (including leaves, stems, rachises, grains and roots). The concentration of Cd in organs in the same treatment followed the order of roots > stems > rachises > others in H soil and the order of roots > stems > other parts in W soil. The concentration of Cd in organs in H soil were higher than that in the corresponding organs in W soil (Fig. 1a-b).

The treatments of F5D3 and F5D7 significantly affected the concentrations of As in rice organs, increase drainage days led to decrease in the As concentrations in all organs. Under the same water management condition, the order of As concentration in each organ was roots > leaves > stems > rachises > grains in both soils (Fig. 1c-d).

Multiple regression analysis showed that the variations in the Cd and As concentration in roots could explain 98% ($R^2 = 0.98$) and 93% ($R^2 = 0.93$) of the observed variations in the Cd and As concentration in grains, respectively (Table 1).

Table 1

Cd in grains	SRF	R^2	P	B	β	Multiple linear regression equation
	Cd in roots	0.98	0.00	0.08	0.99	$Y = -0.80 + 0.08X$
As in grains						
	As in roots	0.93	0.00	0.01	0.97	$Y = -0.25 + 0.01X$

The multiple regression analysis was used to check the main factor influencing the concentration of Cd or As in grains. The grains Cd or As concentration was used as the dependent variable. Variables were Cd and As concentrations in different organs, soil solution Cd and As concentrations, Eh, and pH. The optimum regression equation is deduced by the stepwise method. Significant relative factor is abbreviated as SRF.

Soil solution Cd and As concentrations, soil Eh, and pH and relationships between them

In each corresponding wet-dry alternation cycle treatment, the concentrations of Cd in the F5D3-D3, F5D5-D5, and F5D7-D7 were higher than that in the F5D3-F5, F5D5-F5, and F5D7-F5 in both soils, respectively. In H soil, the soil Cd concentrations followed the order of F5D3-F5 < F5D5-F5 < F5D7-F5, as well as the Cd concentrations in the F5D3-D3, F5D5-D5, and F5D7-D7. However, there was nearly no difference among the treatments in W soil, except the Flooded treatment with the lowest value (Fig. 2a). The concentrations of As in the H soil solution were higher than those in the W soil in all water management treatments. The concentration of As in the F5D3-F5, F5D5-F5, and F5D7-F5 was higher than that in the F5D3-D3, F5D5-D5, and F5D7-D7 in both soils, respectively (Fig. 2b).

Soil pH values in H soil were greater than those in W soil in all water treatments. The highest (6.96 in soil W, 7.2 in soil H) and lowest (6.3 in soil W, 6.7 in soil H) values of soil pH were observed in the Flooded and Drained treatments in both soils, respectively. In the wet-dry alternation cycle treatments, soil pH followed the order of F5D3 < F5D5 < F5D7 in both soils (Fig. 2c). The highest and lowest values of soil Eh were detected in the Drained and Flooded treatments, respectively. In the Flooded, F5D3-F5, F5D5-F5, and F5D7-F5 treatments, the soil Eh ranged from -200 to 0 mV. However, the soil Eh showed positive values (ranging approximately from 0 to 300 mV) in the Drained treatment as well as in F5D3-D3, F5D5-D5, and

F5D7-D7. In F5D3-D3, F5D5-D5, and F5D7-D7, more drainage days corresponded to a higher soil Eh (Fig. 2d).

The variations in Cd concentration were positively and negatively correlated with the soil Eh and pH in both soils, respectively; with the variations in soil Eh explaining 89% and 99% of the variations in the solution Cd in W soil and H soil, respectively; and the variations in soil pH explaining 98% and 94% of the variations in the solution Cd in W soil and H soil, respectively. The relations between soil solution Cd and Eh in H soil, soil solution Cd and pH in W soil were more significant. When soil Eh was above 300 mV, the Cd concentrations reached 1.13 and 13.7 $\mu\text{g L}^{-1}$ in W soil and H soil, respectively. The concentration of Cd was negligible in the soil solution with pH above 6.9 (W soil) and 7.2 (H soil) (Fig. 3a-b).

The variations in the solution As were negatively but positively correlated with Eh and pH in both soils, respectively; with the variations in soil Eh and pH explaining 99% of the variations in solution As in both soils. Very low concentrations (2.4 $\mu\text{g L}^{-1}$) in solution As were demonstrated for Eh above 100 mV. The solution As in H soil was greater than that in W soil under the same Eh condition. The patterns of relationships between the solution As and pH showed great discrepancy. Higher values of soil solution As concentration were observed when pH was above 6.9 in W soil and 7.2 in H soil (Fig. 3c-d).

Amino acid profiles in rice organs

The total concentration of essential amino acid (EAA, including Try, Met, Thr, Leu, Val, Phe, Iso, and Lys) in rice organs followed the order of stems > husks > roots under the same water treatment condition. Thr and Val were detected as the dominant EAAs in both husks and stems, and Val and Lys were dominant in roots. The total concentration of the EAA decreased in husks and roots in the F5D3 treatment in W soil. However, the total concentration of the EAAs in stems increased gradually in the order of Flooded < F5D3 < Drained treatments in W soil, as well as that of organs in H soil (Fig. 4a-c).

The higher values of the total non-essential amino acid (NEAA) concentration were detected in the Drained treatment in both soils. The F5D3 treatment reduced the total NEAA in both husks and roots in W soil and only in roots in H soil. The total NEAA concentration increased gradually in treatments in the order of Flooded < F5D3 < Drained in the husks in H soil and in the stems in both soils. The total NEAA concentration exhibited the order of husks > stems > roots under the same water management condition. Asp-a (Asparagine) was found as the main NEAA in husks and roots. However, the concentration of Glu-i was the highest in stems (Fig. 4d-f).

The main factors influencing the EAAs and NEAAs in rice organs

For EAAs in husks, the variation in rachises As concentration could explain 78% and 81% of the variations in Lev and Phe, respectively. The variations in the soil solution As concentration was the main influencing factor for Try, explaining 79% of the variance in Try. For NEAAs, three types of NEAAs were affected by one or two variables, with the variations in rachises As concentration and Eh explaining 68% and 83% of the changes in Cys and Ser concentration, respectively. The changes in variables could explain 99% of the

variations in Tyr concentration, and the variation in rachises As concentration was the most influential factor, followed by the leaves As concentration.

For EAAs in stems, the variations in the rachises As concentration was the significant influential factor for the variations in Phe, Thr, and Val concentrations and could explain 80–84% of variations in them. As concentration in rachises and stems were the main effect factors for the variation in Lys concentration and could explain 98% of the changes in Lys. For NEAAs, the variations in Arg, Glu-i, Gly, and His concentrations could be explained by the variations in the rachises Cd concentration (96%) and Eh (83%) and the rachises As concentration (92%) and Eh (88%), respectively. The variations in the rachises As and Eh explained 96% and 95% of the variations in Glu-a and Tyr concentration, respectively. The variations in the rachises As and leaves Cd concentrations, the solution As concentration and pH, and the rachises As concentration and pH could explain more than 98% of the variations in Asp, Prd, and Sar/Ala concentrations, respectively. The variation in the rachises As concentration was the most influential factor for the variations in Asp, Glu-a, Sar/Ala, and Tyr concentrations.

For EAAs in roots, the variation in Met could be explained by the variation in the rachises As concentration by 83%. For NAEs, the variation in the roots Cd concentration could explain 77% of the Asp-a concentration variations, and 70% of the variation in the Asp-t concentration could be explained by the variation in the rachises Cd concentration (Table 2).

Table 2

Husks			SRF	R ²	P	B	β	Multiple linear regression equation
	EAAs	Lev	As in rachises	0.78	0.02	-65.7	-0.88	Y = 848 – 65.7X
		Phe	As in rachises	0.81	0.01	-95.5	-0.90	Y = 1228 – 95.5X
		Try	As in solution	0.79	0.02	0.01	0.89	Y = 0.30 + 0.01X
	NEAAs	Cys	As in rachises	0.68	0.04	-16.7	-0.82	Y = 180 – 16.7X
		Ser	Eh	0.83	0.01	4.21	0.91	Y = 5890 + 4.21X
		Tyr		0.99				Y = 957 – 63.2 × 1 + 7.22 × 2
			As in rachises		0.00	-63.2	-1.34	
Stems	EAAs		As in leaves		0.02	7.22	0.44	
		Phe	As in rachises	0.84	0.01	-453	-0.92	Y = 3555 – 453X
		Thr	As in rachises	0.80	0.02	-638	-0.90	Y = 5300 – 638X
		Val	As in rachises	0.84	0.01	-503	-0.92	Y = 5627 – 503X
		Lys		0.98				
			As in rachises		0.00	-273	-1.9	Y = 1250 – 273 × 1 + 91.1 × 2
	NEAAs		As in stems		0.02	91.1	1.03	
		Arg	Cd in rachises	0.96	0.00	293	0.98	Y = 767 + 293X
		Glu-i	Eh	0.83	0.01	13.8	0.91	Y = 12833 + 13.8X
		Gly	As in rachises	0.92	0.00	-80.3	-0.96	Y = 602 – 80.3X
		His	Eh	0.88	0.01	1.23	0.94	Y = 1087 + 1.23X
		Asp-a		0.98				Y = 3038 – 332 × 1 + 58.6 × 2

Husks			SRF	R ²	P	B	β	Multiple linear regression equation
			As in rachises		0.01	-332	-0.65	
			Cd in leaves		0.04	58.6	0.43	
Glu-a				0.96				$Y = 9294 - 1219 \times 1 - 8.31 \times 2$
			As in rachises		0.01	-1912	-2.67	
			Eh		0.03	-8.31	-1.84	
Prd				0.99				$Y = -1332 - 11.6 \times 1 + 506 \times 2$
			As in solution		0.00	-11.6	-1.39	
			pH		0.01	506	0.45	
Sar/Ala				0.98				$Y = -7137 - 972 \times 1 + 1791 \times 2$
			As in rachises		0.00	-972	-1.28	
			pH		0.03	1791	0.49	
Tyr				0.95				$Y = 2158 - 542 \times 1 - 2.07 \times 2$
			As in rachises		0.01	-542	-2.27	
			Eh		0.04	-2.07	-1.38	
Roots	EAA	Met	As in rachises	0.83	0.01	13.1	0.91	$Y = 44 + 13.1X$
	NEAA	Asp-a	Cd in roots	0.77	0.02	84.2	0.88	$Y = 2741 + 84.2X$
		Asp-t	Cd in rachises	0.70	0.04	21.2	0.84	$Y = 1151 + 21.2X$

The multiple regression analysis was used to check the main factors influencing EAAs and NEAAs in the plant organs (husks, stems, and roots). The concentrations of various EAAs or NEAAs in the plant organs have been used as dependent variables. Variables were organs Cd and As concentrations, soil solution Cd

and As concentrations, Eh, and pH. The optimum regression equation is deduced by the stepwise method. Significant relative factors is abbreviated as SRF.

Discussion

Effects of different dry-wet alternation regimes on Cd and As uptake in rice

Dry-wet alternation regimes had marked but opposite effects on Cd and As accumulation in rice. More drainage days led to higher Cd contents but lower As contents in both soils, these results are similar to those reported by Arao et al. (2009)¹³, Hu et al. (2013)¹⁴, Wan et al. (2019)¹⁸, and Mlangeni et al. (2020)¹⁹. The magnitude of distribution of Cd and As concentrations in rice in both soils was in the following order: roots > other aboveground organs > grains, as also observed by Xie et al. (2015)²⁰ report. The Cd concentrations in individual organs in the Drained treatment were 1.4–24.8 (in soil W) and 5.2–778 (in soil H) times as high as those in the Flooded treatment; however, the As concentration in the Flooded treatment was 3.5–13.0 (in soil W) and 6.8–29.5 (in soil H) times as high as those in the Drained treatment. This clearly demonstrated that rice Cd accumulation was more sensitive than As accumulation to water regimes in this study, as Hu et al. (2013)²¹ also reported. In grains, the F5D3, F5D5, and F5D7 treatments separately reduced Cd concentrations by 80.0%, 64.3%, and 18.5% in W soil and 90.8%, 82.4%, and 76.1% in H soil relative to those in the Drained treatment, respectively. In addition, As concentrations were reduced by 77.4%, 77.8%, and 86.7% in W soil and 73.1%, 77.2%, and 80.6% in H soil, respectively, relative to those in the Flooded treatment. Cd and As accumulations in grains was likely related to the translocation of Cd and As from one organ to another (Fig. S1). The accumulation of Cd in rice grains is determined by xylem-mediated Cd translocation from root to shoot and phloem-mediated Cd transport^{22,23}. Drainage condition decreased the TFs of Cd from rachises to grains but increased the TFs of Cd from stems to rachises and the TFs of Cd from roots to stems (Fig. S1a). We also found that the variation in the Cd concentration in roots could explain 98% of the observed variation in the Cd concentration in grains in the present study, indicating that inhibition of the TFs of Cd from stems to rachises and restraint of the Cd accumulation in roots could further reduce Cd content in grains under drainage conditions. Flooding significantly increased the TFs of As from roots to stems in W soil and also favored the TFs of As from rachises to grains. The TFs of As from rachises to grains were higher than other TFs in the same treatment (Fig. S1b). Additionally, the multiple stepwise regressions showed that the variation in the As concentration in roots could explain 93% of the obtained variation in the As concentration in grains. Thus, the efficient reduction of As content in grains could be achieved by restraining As accumulation in roots and inhibiting As transportation from roots to shoots and from rachises to grains under flooding conditions. The F5D3 treatment was the most efficient in reducing both Cd and As content concurrently in both soils in the present study. Honmat et al. (2016)¹⁵ found that the 3 d Flooded and 5 d Drained treatment was the most efficient for the concurrent reduction of Cd and As in grains. Clearly, the optimal wet-dry alternation cycles identified through one or two studies is not applicable across all different soil types (e.g., different pH, organic matter, cation exchange capacity and

different content of Cd and As in soils. It is relatively difficult to reduce Cd and As simultaneously in rice grains through water regimes alone in soil polluted with both Cd and As.

The fact that Cd and As accumulation in rice was higher in H soil than in W soil was probably owing to the higher soil total Cd and As in H soil than in W soil. Recent studies have indicated that some soils are enriched in Cd but with low bioavailability. In acidic soils with relatively low total Cd, the grown rice plants absorbed a considerable amount of Cd and accumulated higher Cd in their grains²⁴. Based on soil geochemical survey data with GIS, Xia et al. (2019)²⁵ also noted that the spatial patterns of Cd in rice were not consistent with the soil Cd levels. The absorption of Cd by rice also depends on the rice cultivar²⁶. These quite distinct results imply that the soil Cd and As pollution evaluation methods need to continue improving through best practices and consideration of the bioavailability of Cd and As.

Effects of dry-wet alternation regimes on Cd and As content in the soil solution

The soil solution Cd concentrations followed the order of Flooded < F5D3 < F5D5 < F5D7 < Drained treatment in both soils (Fig. 2a), and the soil solution Cd in H soil were higher than those in W soil, which were likely dependent on the total Cd in soil, as we observed higher soil total Cd concentrations in H soil than in W soil. The concentration of dissolved Cd in the soil solution is directly correlated with the amount of absorption by plants²⁷. Dry-wet alternation regimes could lead to changes in soil pH and Eh (Fig. 2c-d), which further control the forms of Cd in soil and thereby affect its bioavailability¹⁵. The patterns of variations in the soil solution Cd concentrations were negatively correlated with soil pH, as also observed by Han et al. (2018)²⁸. The relationship between soil solution Cd concentration and pH was more significant in W soil. Studies reported that soil pH increased under flooding conditions owing to greater cation adsorption^{29,30}. The flooding treatment decreased the soil-available Cd concentration owing to greater immobilization of Cd in flooded soils as a result of higher soil pH³¹. Soil pH also alters Cd availability by affecting surface charge (which is pH dependent) and thereby altering their affinity to Cd sorption sites³². Redox changes in paddies substantially affect the phases of Cd and As^{33,34}. The variations in solution Cd were positively correlated with Eh in both soils, in agreement with the results of Han et al. (2018)²⁸. The relation between soil solution Cd and Eh was more significant in H soil. Previous studies have reported that under low Eh (flooding conditions), NO_3^- , SO_4^{2-} , Fe(III), and Mn(III/VI) receive electrons via soil microbe respiration³⁵ and are reduced to NO_2^- , S^{2-} , Fe^{2+} , and Mn^{2+} , respectively. The reductions in NO_3^- and SO_4^{2-} could lead to decreases in water-soluble Cd- SO_4 and Cd- NO_3 , thereby reducing soil solution Cd. Additionally, soil S will potentially be reduced to S^{2-} under flooding conditions, reacting with Cd to form CdS, which can reduce the aqueous solubility of Cd¹³. Under aerobic conditions, the availability of Cd increases because of the oxidation of CdS to Cd^{2+} and SO_4^{2-} ³⁶. However, in this study, there was nearly no difference in the soil solution Cd concentrations among treatments in soil W, except for the Flooded treatment having the lowest value, which was possibly affected by variations in other factors, such as organic matter, clay mineral content, Fe/Mn oxide content, mineral nutrients, and fertilizer^{37,38}.

In treatments involving anaerobic conditions, soil solution As concentration was higher than in treatments involving aerobic condition in both soils (Fig. 2b), and they were higher in H soil than in W soil. Positive and negative relationships existed between the soil solution As and pH and Eh, respectively, in both soils in this study, as in a previous study³⁹. Thus, alternating between flooding and drainage in paddy fields plays an important role in the variation in soil solution As owing to the effects on soil pH and Eh, even in terms of As availability to rice plants^{5,1}. Therefore, the most efficient means to alleviate As accumulation in rice would be to maintain aerobic conditions during the growth season⁴⁰. To evaluate the degree of trade-off between soil solution As and Cd concentrations and identify the optimal water management strategy to simultaneously minimize the concentrations of both, we calculated the “trade-off value” in accordance with a report by Honma et al. (2016)¹⁵. The minimum trade-off value was observed in the F5D3 treatments in both soils, with a value of 0.86, corresponding to an Eh of -20 and pH of 6.65, in soil W, and a value of 0.26, corresponding to an Eh of -8 and pH of 6.89, in soil H. Thus, the F5D3 water treatment was most effective for the simultaneous reduction of total dissolved Cd and As for both soils in this study.

Effects of water management regimes on AAs of organs and factors associated with the variation in individual AAs

Drainage measures led to increases in the total concentration of AAs in nearly all detected organs in both soils. Thr and Val were detected as the dominant EAAs in both husks and stems, whereas Val and Lys were dominant in the roots. Studies reported that Val (one of the branch-chain amino acids) and Lys are controlled by complex systems, and these amino acids are usually used as substrates to produce secondary metabolites in high plants⁴¹. Lys and branched-chain amino acids play a vital role in the plant response to various abiotic stresses⁴². Asp-a and Glu-i displayed the highest levels among the NEAAs in husks and stems, respectively. Multiple regression analysis showed that four types of EAAs (Phe, Thr, Val, and Lys) and five types of NEAAs (Gly, Asp-a, Glu-a, Sar/Ala, and Tyr) in stems were negatively affected by the variation in rachises As concentration; furthermore, the variation in rachises As concentration could explain 80–98% of the variations in the amino acids. The variation in rachises As concentration also negatively affected the variations in Lev, Phe, Cys, and Tyr concentrations in husks, which could explain 68%-99% of their variations. Dwivedi et al. (2012)⁴³ demonstrated that As accumulation in rice grain changed EAAs and NEAAs differentially, grain As accumulation negatively correlated with EAAs, especially in rice with high As accumulating varieties. This probably means that water management regimes regulated the synthesis of AAs in rice organs by affecting the accumulation of As in the rachises. Previous studies have reported that plant defense systems are activated under heavy metal stress, improving the synthesis of defense-related AAs. Some types of AAs are involved in metal-ion chelation⁴⁴ as Pro is often detected in plants suffering from heavy-metal stress⁴⁵. In the F5D3 treatment, the total concentration of EAAs and NEAAs in husks and total EAAs in roots in W soil, as well as the total concentration of the NEAAs in roots in both soils, were lower compared with those of the Flooded and Drained treatments, and lower concentrations of Cd and As in rice organs were also detected in the F5D3 treatment compared with those detected in the Flooded or Drained treatments, the variations in As

concentrations (in rachises) and Cd concentrations (in roots and rachises) significantly influenced the variations in some types of AAs concentrations in husks or roots (Table 2), indicating that it might not be necessary to synthesize additional AAs to chelate metal ions in the F5D3 treatment. Further studies should be based on what we have demonstrated here and further elucidate the mechanisms underlying the interaction between water management regimes and Cd and As content in rice organs with respect to AA synthesis.

This study showed that total concentration of AAs in husks was higher than that in stems and roots under the same water treatment conditions. Most of the materials needed for grain filling come from the photosynthesis of the husk, and the development of the husk directly affects the grain filling; thus, AAs in the husk certainly regulate the levels of AAs in grains. The different responses of AA synthesis in rice organs (especially rice husk) to water management regimes in Cd and As polluted soils may be helpful in maintaining the metabolic balance related to the yield and quality of rice.

Conclusions

Water management influences the Cd and As bioavailability in paddy soils with different contamination levels, affecting Cd and As concentrations in rice. Taken as a whole, results showed that the response of rice Cd accumulations was more sensitive than that of As accumulation to water regimes, and the wet-dry alternation cycles were more effective in reducing grain's Cd accumulation in W soil (reductions ranged from 18.8%-80.8%). The Cd and As accumulations in rice organs were affected by the content of Cd and As in soil and in the soil solution. The variations in concentrations in root's Cd and As primarily determined the variations in grains Cd and As concentrations. The rachises As concentration negatively affected most of the AAs content. In brief, the wet-dry alternation cycle of the 5 d flooding followed by 3 d drainage from elongation stage to grain filling stage of rice was identified as the optimal wet-dry alternation measure for simultaneously reducing the Cd and As accumulations in rice in both soils. Water management regimes regulated the synthesis of AAs in rice organs by affecting the accumulation of As in rachises.

Declarations

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Figures

Figure 1

Effects of water management regimes on Cd concentrations in shoots (grains, rachises, leaves, and stems) (a) and roots (b), and As concentrations in shoots (grains, rachises, leaves, and stems) (c) and roots (d). The multiple comparison was conducted among different treatments within the same organ ($\alpha=0.05$). The plotted columns are mean $\pm$ SD ($n=4$).

Figure 2

Effects of water management regimes on the concentration of Cd (a) and As (b) in the soil solution, soil Eh (c), and pH (d). F5D3-F5, F5D5-F5, and F5D7-F5 mean the fifth day of flooding in F5D3, F5D5, and F5D7 treatment; F5D3-D3, F5D5-D5, and F5D7-D7 means the third day, the fifth day, and the seventh day of drainage in F5D3, F5D5, and F5D7 treatment, respectively. The multiple comparison was conducted among different treatments within the same soil ($\alpha=0.05$). The plotted columns are mean $\pm$ SD ($n=4$).

Figure 3

Relationships between soil solution Cd concentration and Eh (a), pH (b) and between solution As concentration and Eh (c) and pH (d).

Figure 4

Effects of water management regimes on the concentrations of the essential amino acids (EAAs, Tryptophan [Try], Methionine [Met], Threonine [Thr], Leucine [Leu], Valine [Val], Phenylalanine [Phe], Isoleucine [Iso], and Lysine [Lys]) and the non-essential amino acids (NEAAs, Cysteine [Cys], Alanine [Ala], Serine [Ser], Arginine [Arg], Glutamine [Glu-i], Glycine [Gly], Asparagine [Asp-a], Glutamate [Glu-a], Tyrosine [Tyr], Histidine [His], Proline [Pro], and Aspartate [Asp-t]) in the plant organs in two soils. (a), (b), and (c)

represent the concentrations of EAAs in husks, stems, and roots, respectively. (c), (d), and (f) represent the concentrations of NEAAs in husks, stems, and roots, respectively.

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